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(54) Title: METHODS FOR GENERATING GENETICALLY ALTERED ANTIBODY-PRODUCING CELL LINES WITH IMPROVED ANTIBODY CHARACTERISTICS

(57) Abstract: Dominant negative alleles of human mismatch repair genes can be used to generate hypermutable cells and organisms. By introducing these genes into cells and transgenic animals, new cell lines and animal varieties with novel and useful properties can be prepared more efficiently than by relying on the natural rate of mutation. These methods are useful for generating genetic diversity within immunoglobulin genes directed against an antigen of interest to produce altered antibodies with enhanced biochemical activity. Moreover, these methods are useful for generating antibody-producing cells with increased level of antibody production.

**METHODS FOR GENERATING GENETICALLY ALTERED ANTIBODY-
PRODUCING CELL LINES WITH IMPROVED ANTIBODY
CHARACTERISTICS**

5 TECHNICAL FIELD OF THE INVENTION

The invention is related to the area of antibody maturation and cellular production. In particular, it is related to the field of mutagenesis.

BACKGROUND OF THE INVENTION

10 The use of antibodies to block the activity of foreign and/or endogenous polypeptides provides an effective and selective strategy for treating the underlying cause of disease. In particular is the use of monoclonal antibodies (MAb) as effective therapeutics such as the FDA approved ReoPro (Glaser, V. (1996) Can ReoPro repolish tarnished monoclonal therapeutics? *Nat. Biotechnol.* 14:1216-1217), an anti-platelet MAb
15 from Centocor; Herceptin (Weiner, L.M. (1999) Monoclonal antibody therapy of cancer. *Semin. Oncol.* 26:43-51), an anti-Her2/neu MAb from Genentech; and Synagis (Saez-Llorens, X.E., *et al.* (1998) Safety and pharmacokinetics of an intramuscular humanized monoclonal antibody to respiratory syncytial virus in premature infants and infants with bronchopulmonary dysplasia. *Pediat. Infect. Dis. J.* 17:787-791), an anti-respiratory
20 syncytial virus MAb produced by Medimmune.

Standard methods for generating MAbs against candidate protein targets are known by those skilled in the art. Briefly, rodents such as mice or rats are injected with a purified antigen in the presence of adjuvant to generate an immune response (Shield, C.F., *et al.* (1996) A cost-effective analysis of OKT3 induction therapy in cadaveric kidney
25 transplantation. *Am. J. Kidney Dis.* 27:855-864). Rodents with positive immune sera are sacrificed and splenocytes are isolated. Isolated splenocytes are fused to melanomas to produce immortalized cell lines that are then screened for antibody production. Positive lines are isolated and characterized for antibody production. The direct use of rodent MAbs as human therapeutic agents were confounded by the fact that human anti-rodent
30 antibody (HARA) responses occurred in a significant number of patients treated with the rodent-derived antibody (Khazaeli, M.B., *et al.*, (1994) Human immune response to monoclonal antibodies. *J. Immunother.* 15:42-52). In order to circumvent the problem of HARA, the grafting of the complementarity determining regions (CDRs), which are the

critical motifs found within the heavy and light chain variable regions of the immunoglobulin (Ig) subunits making up the antigen binding domain, onto a human antibody backbone found these chimeric molecules are able to retain their binding activity to antigen while lacking the HARA response (Emery, S.C., and Harris, W.J. "Strategies for humanizing antibodies" In: ANTIBODY ENGINEERING C.A.K. Borrebaeck (Ed.) Oxford University Press, N.Y. 1995. pp. 159-183. A common problem that exists during the "humanization" of rodent-derived MAbs (referred to hereon as HAb) is the loss of binding affinity due to conformational changes in the 3 dimensional structure of the CDR domain upon grafting onto the human Ig backbone (U.S. Patent No. 5,530,101 to Queen *et al.*). To overcome this problem, additional HAb vectors are usually needed to be engineered by inserting or deleting additional amino acid residues within the framework region and/or within the CDR coding region itself in order to recreate high affinity HAb (U.S. Patent No. 5,530,101 to Queen *et al.*). This process is a very time consuming procedure that involves the use of expensive computer modeling programs to predict changes that may lead to a high affinity HAb. In some instances the affinity of the HAb is never restored to that of the MAb, rendering them of little therapeutic use.

Another problem that exists in antibody engineering is the generation of stable, high yielding producer cell lines that is required for manufacturing of the molecule for clinical materials. Several strategies have been adopted in standard practice by those skilled in the art to circumvent this problem. One method is the use of Chinese Hamster Ovary (CHO) cells transfected with exogenous Ig fusion genes containing the grafted human light and heavy chains to produce whole antibodies or single chain antibodies, which are a chimeric molecule containing both light and heavy chains that form an antigen-binding polypeptide (Reff, M.E. (1993) High-level production of recombinant immunoglobulins in mammalian cells. *Curr. Opin. Biotechnol.* 4:573-576). Another method employs the use of human lymphocytes derived from transgenic mice containing a human grafted immune system or transgenic mice containing a human Ig gene repertoire. Yet another method employs the use of monkeys to produce primate MAbs, which have been reported to lack a human anti-monkey response (Neuberger, M., and Gruggermann, M. (1997) Monoclonal antibodies. Mice perform a human repertoire. *Nature* 386:25-26). In all cases, the generation of a cell line that is capable of generating sufficient amounts of high affinity antibody poses a major limitation for producing sufficient materials for clinical studies. Because of these limitations, the utility of other recombinant systems such

as plants are currently being explored as systems that will lead to the stable, high-level production of humanized antibodies (Fiedler, U., and Conrad, U. (1995) High-level production and long-term storage of engineered antibodies in transgenic tobacco seeds. *Bio/Technology* 13:1090-1093).

5 A method for generating diverse antibody sequences within the variable domain that results in HAbs and MAbs with high binding affinities to antigens would be useful for the creation of more potent therapeutic and diagnostic reagents respectively. Moreover, the generation of randomly altered nucleotide and polypeptide residues throughout an
10 beneficial pharmacokinetic properties. The invention described herein is directed to the use of random genetic mutation throughout an antibody structure *in vivo* by blocking the endogenous mismatch repair (MMR) activity of a host cell producing immunoglobulins that encode biochemically active antibodies. The invention also relates to methods for repeated *in vivo* genetic alterations and selection for antibodies with enhanced binding and
15 pharmacokinetic profiles.

 In addition, the ability to develop genetically altered host cells that are capable of secreting increased amounts of antibody will also provide a valuable method for creating cell hosts for product development. The invention described herein is directed to the creation of genetically altered cell hosts with increased antibody production via the
20 blockade of MMR.

 The invention facilitates the generation of high affinity antibodies and the production of cell lines with elevated levels of antibody production. Other advantages of the present invention are described in the examples and figures described herein.

SUMMARY OF THE INVENTION

25 The invention provides methods for generating genetically altered antibodies (including single chain molecules) and antibody producing cell hosts *in vitro* and *in vivo*, whereby the antibody possess a desired biochemical property(s), such as, but not limited to, increased antigen binding, increased gene expression, and/or enhanced extracellular secretion by the cell host. One method for identifying antibodies with increased binding
30 activity or cells with increased antibody production is through the screening of MMR defective antibody producing cell clones that produce molecules with enhanced binding properties or clones that have been genetically altered to produce enhanced amounts of antibody product.

The antibody producing cells suitable for use in the invention include, but are not limited to rodent, primate, or human hybridomas or lymphoblastoids; mammalian cells transfected and expressing exogenous Ig subunits or chimeric single chain molecules; plant cells, yeast or bacteria transfected and expressing exogenous Ig subunits or chimeric single chain molecules.

Thus, the invention provides methods for making hypermutable antibody-producing cells by introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into cells that are capable of producing antibodies. The cells that are capable of producing antibodies include cells that naturally produce antibodies, and cells that are engineered to produce antibodies through the introduction of immunoglobulin encoding sequences. Conveniently, the introduction of polynucleotide sequences into cells is accomplished by transfection.

The invention also provides methods of making hypermutable antibody producing cells by introducing a dominant negative mismatch repair (MMR) gene such as *PMS2* (preferably human *PMS2*), *MLH1*, *PMS1*, *MSH2*, or *MSH2* into cells that are capable of producing antibodies. The dominant negative allele of a mismatch repair gene may be a truncation mutation of a mismatch repair gene (preferably a truncation mutation at codon 134, or a thymidine at nucleotide 424 of wild-type *PMS2*). The invention also provides methods in which mismatch repair gene activity is suppressed. This may be accomplished, for example, using antisense molecules directed against the mismatch repair gene or transcripts.

Other embodiments of the invention provide methods for making a hypermutable antibody producing cells by introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into fertilized eggs of animals. These methods may also include subsequently implanting the eggs into pseudo-pregnant females whereby the fertilized eggs develop into a mature transgenic animal. The mismatch repair genes may include, for example, *PMS2* (preferably human *PMS2*), *MLH1*, *PMS1*, *MSH2*, or *MSH2*. The dominant negative allele of a mismatch repair gene may be a truncation mutation of a mismatch repair gene (preferably a truncation mutation at codon 134, or a thymidine at nucleotide 424 of wild-type *PMS2*).

The invention further provides homogeneous compositions of cultured, hypermutable, mammalian cells that are capable of producing antibodies and contain a dominant negative allele of a mismatch repair gene. The mismatch repair genes may

include, for example, *PMS2* (preferably human *PMS2*), *MLH1*, *PMS1*, *MSH2*, or *MSH2*. The dominant negative allele of a mismatch repair gene may be a truncation mutation of a mismatch repair gene (preferably a truncation mutation at codon 134, or a thymidine at nucleotide 424 of wild-type *PMS2*). The cells of the culture may contain *PMS2*, (preferably human *PMS2*), *MLH1*, or *PMS1*; or express a human *mutL* homolog, or the first 133 amino acids of hPMS2.

The invention further provides methods for generating a mutation in an immunoglobulin gene of interest by culturing an immunoglobulin producing cell selected for an immunoglobulin of interest wherein the cell contains a dominant negative allele of a mismatch repair gene. The properties of the immunoglobulin produced from the cells can be assayed to ascertain whether the immunoglobulin gene harbors a mutation. The assay may be directed to analyzing a polynucleotide encoding the immunoglobulin, or may be directed to the immunoglobulin polypeptide itself.

The invention also provides methods for generating a mutation in a gene affecting antibody production in an antibody-producing cell by culturing the cell expressing a dominant negative allele of a mismatch repair gene, and testing the cell to determine whether the cell harbors mutations within the gene of interest, such that a new biochemical feature (*e.g.*, over-expression and/or secretion of immunoglobulin products) is generated. The testing may include analysis of the steady state expression of the immunoglobulin gene of interest, and/or analysis of the amount of secreted protein encoded by the immunoglobulin gene of interest. The invention also embraces prokaryotic and eukaryotic transgenic cells made by this process, including cells from rodents, non-human primates and humans.

Other aspects of the invention encompass methods of reversibly altering the hypermutability of an antibody producing cell, in which an inducible vector containing a dominant negative allele of a mismatch repair gene operably linked to an inducible promoter is introduced into an antibody-producing cell. The cell is treated with an inducing agent to express the dominant negative mismatch repair gene (which can be *PMS2* (preferably human *PMS2*), *MLH1*, or *PMS1*). Alternatively, the cell may be induced to express a human *mutL* homolog or the first 133 amino acids of hPMS2. In another embodiment, the cells may be rendered capable of producing antibodies by co-transfecting a preselected immunoglobulin gene of interest. The immunoglobulin genes of the hypermutable cells, or the proteins produced by these methods may be analyzed for desired

properties, and induction may be stopped such that the genetic stability of the host cell is restored.

The invention also embraces methods of producing genetically altered antibodies by transfecting a polynucleotide encoding an immunoglobulin protein into a cell containing a dominant negative mismatch repair gene (either naturally or in which the dominant negative mismatch repair gene was introduced into the cell), culturing the cell to allow the immunoglobulin gene to become mutated and produce a mutant immunoglobulin, screening for a desirable property of said mutant immunoglobulin protein, isolating the polynucleotide molecule encoding the selected mutant immunoglobulin possessing the desired property, and transfecting said mutant polynucleotide into a genetically stable cell, such that the mutant antibody is consistently produced without further genetic alteration. The dominant negative mismatch repair gene may be *PMS2* (preferably human *PMS2*), *MLH1*, or *PMS1*. Alternatively, the cell may express a human *mutL* homolog or the first 133 amino acids of hPMS2.

The invention further provides methods for generating genetically altered cell lines that express enhanced amounts of an antigen binding polypeptide. These antigen-binding polypeptides may be, for example, immunoglobulins. The methods of the invention also include methods for generating genetically altered cell lines that secrete enhanced amounts of an antigen binding polypeptide. The cell lines are rendered hypermutable by dominant negative mismatch repair genes that provide an enhanced rate of genetic hypermutation in a cell producing antigen-binding polypeptides such as antibodies. Such cells include, but are not limited to hybridomas. Expression of enhanced amounts of antigen binding polypeptides may be through enhanced transcription or translation of the polynucleotides encoding the antigen binding polypeptides, or through the enhanced secretion of the antigen binding polypeptides, for example.

Methods are also provided for creating genetically altered antibodies *in vivo* by blocking the MMR activity of the cell host, or by transfecting genes encoding for immunoglobulin in a MMR defective cell host.

Antibodies with increased binding properties to an antigen due to genetic changes within the variable domain are provided in methods of the invention that block endogenous MMR of the cell host. Antibodies with increased binding properties to an antigen due to genetic changes within the CDR regions within the light and/or heavy

chains are also provided in methods of the invention that block endogenous MMR of the cell host.

The invention provides methods of creating genetically altered antibodies in MMR defective Ab producer cell lines with enhanced pharmacokinetic properties in host
5 organisms including but not limited to rodents, primates, and man.

These and other aspects of the invention are provided by one or more of the embodiments described below. In one embodiment of the invention, a method for making an antibody producing cell line hypermutable is provided. A polynucleotide encoding a dominant negative allele of a MMR gene is introduced into an antibody-producing cell.
10 The cell becomes hypermutable as a result of the introduction of the gene.

In another embodiment of the invention, a method is provided for introducing a mutation into an endogenous gene encoding for an immunoglobulin polypeptide or a single chain antibody. A polynucleotide encoding a dominant negative allele of a MMR gene is introduced into a cell. The cell becomes hypermutable as a result of the
15 introduction and expression of the MMR gene allele. The cell further comprises an immunoglobulin gene of interest. The cell is grown and tested to determine whether the gene encoding for an immunoglobulin or a single chain antibody of interest harbors a mutation. In another aspect of the invention, the gene encoding the mutated immunoglobulin polypeptide or single chain antibody may be isolated and expressed in a
20 genetically stable cell. In a preferred embodiment, the mutated antibody is screened for at least one desirable property such as, but not limited to, enhanced binding characteristics.

In another embodiment of the invention, a gene or set of genes encoding for Ig light and heavy chains or a combination therein are introduced into a mammalian cell host that is MMR defective. The cell is grown, and clones are analyzed for antibodies with
25 enhanced binding characteristics.

In another embodiment of the invention, a method will be provided for producing new phenotypes of a cell. A polynucleotide encoding a dominant negative allele of a MMR gene is introduced into a cell. The cell becomes hypermutable as a result of the introduction of the gene. The cell is grown. The cell is tested for the expression of new
30 phenotypes where the phenotype is enhanced secretion of a polypeptide.

These and other embodiments of the invention provide the art with methods that can generate enhanced mutability in cells and animals as well as providing cells and

animals harboring potentially useful mutations for the large-scale production of high affinity antibodies with beneficial pharmacokinetic profiles.

BRIEF DESCRIPTION OF THE DRAWINGS

5 **Figure 1.** Hybridoma cells stably expressing PMS2 and PMS134 MMR genes. Shown is steady state mRNA expression of MMR genes transfected into a murine hybridoma cell line. Stable expression was found after 3 months of continuous growth. The (-) lanes represent negative controls where no reverse transcriptase was added, and the (+) lanes represent samples reverse transcribed and PCR amplified for the MMR
10 genes and an internal housekeeping gene as a control.

Figure 2. Creation of genetically hypermutable hybridoma cells. Dominant negative MMR gene alleles were expressed in cells expressing a MMR-sensitive reporter gene. Dominant negative alleles such as PMS134 and the expression of MMR genes from other species results in antibody producer cells with a hypermutable phenotype that can be
15 used to produce genetically altered immunoglobulin genes with enhanced biochemical features as well as lines with increased Ig expression and/or secretion. Values shown represent the amount of converted CPRG substrate which is reflective of the amount of function β -galactosidase contained within the cell from genetic alterations within the pCAR-OF reporter gene. Higher amounts of β -galactosidase activity reflect a higher
20 mutation rate due to defective MMR.

Figure 3. Screening method for identifying antibody-producing cells containing antibodies with increased binding activity and/or increased expression/secretion

Figure 4. Generation of a genetically altered antibody with an increased binding activity. Shown are ELISA values from 96-well plates, screened for antibodies specific to
25 hIgE. Two clones with a high binding value were found in HB134 cultures.

Figure 5. Sequence alteration within variable chain of an antibody (a mutation within the light chain variable region in MMR-defective HB134 antibody producer cells). Arrows indicate the nucleotide at which a mutation occurred in a subset of cells from a clone derived from HB134 cells. Panel A: The change results in a Thr to Ser
30 change within the light chain variable region. The coding sequence is in the antisense direction. Panel B: The change results in a Pro to His change within the light chain variable region.

Figure 6. Generation of MMR-defective clones with enhanced steady state Ig

protein levels. A Western blot of heavy chain immunoglobulins from HB134 clones with high levels of MAb (>500ngs/ml) within the conditioned medium shows that a subset of clones express higher steady state levels of immunoglobulins (Ig). The H36 cell line was used as a control to measure steady state levels in the parental strain. Lane 1: fibroblast
5 cells (negative control); Lane 2: H36 cell; Lane 3: HB134 clone with elevated MAb levels; Lane 4: HB134 clone with elevated MAb levels; Lane 5: HB134 clone with elevated MAb levels.

Methods have been discovered for developing hypermutable antibody-producing cells by taking advantage of the conserved mismatch repair (MMR) process of host cells.
10 Dominant negative alleles of such genes, when introduced into cells or transgenic animals, increase the rate of spontaneous mutations by reducing the effectiveness of DNA repair and thereby render the cells or animals hypermutable. Hypermutable cells or animals can then be utilized to develop new mutations in a gene of interest. Blocking MMR in antibody-producing cells such as but not limited to: hybridomas; mammalian cells transfected with
15 genes encoding for Ig light and heavy chains; mammalian cells transfected with genes encoding for single chain antibodies; eukaryotic cells transfected with Ig genes, can enhance the rate of mutation within these cells leading to clones that have enhanced antibody production and/or cells containing genetically altered antibodies with enhanced biochemical properties such as increased antigen binding. The process of MMR, also
20 called mismatch proofreading, is carried out by protein complexes in cells ranging from bacteria to mammalian cells. A MMR gene is a gene that encodes for one of the proteins of such a mismatch repair complex. Although not wanting to be bound by any particular theory of mechanism of action, a MMR complex is believed to detect distortions of the DNA helix resulting from non-complementary pairing of nucleotide bases. The non-
25 complementary base on the newer DNA strand is excised, and the excised base is replaced with the appropriate base, which is complementary to the older DNA strand. In this way, cells eliminate many mutations that occur as a result of mistakes in DNA replication.

Dominant negative alleles cause a MMR defective phenotype even in the presence of a wild-type allele in the same cell. An example of a dominant negative allele of a MMR
30 gene is the human gene *hPMS2-134*, which carries a truncating mutation at codon 134 (SEQ ID NO:15). The mutation causes the product of this gene to abnormally terminate at the position of the 134th amino acid, resulting in a shortened polypeptide containing the N-terminal 133 amino acids. Such a mutation causes an increase in the rate of mutations,

which accumulate in cells after DNA replication. Expression of a dominant negative allele of a mismatch repair gene results in impairment of mismatch repair activity, even in the presence of the wild-type allele. Any allele which produces such effect can be used in this invention. Dominant negative alleles of a MMR gene can be obtained from the cells of
5 humans, animals, yeast, bacteria, or other organisms. Such alleles can be identified by screening cells for defective MMR activity. Cells from animals or humans with cancer can be screened for defective mismatch repair. Cells from colon cancer patients may be particularly useful. Genomic DNA, cDNA, or mRNA from any cell encoding a MMR protein can be analyzed for variations from the wild type sequence. Dominant negative
10 alleles of a MMR gene can also be created artificially, for example, by producing variants of the *hPMS2-134* allele or other MMR genes. Various techniques of site-directed mutagenesis can be used. The suitability of such alleles, whether natural or artificial, for use in generating hypermutable cells or animals can be evaluated by testing the mismatch repair activity caused by the allele in the presence of one or more wild-type alleles, to
15 determine if it is a dominant negative allele.

A cell or an animal into which a dominant negative allele of a mismatch repair gene has been introduced will become hypermutable. This means that the spontaneous mutation rate of such cells or animals is elevated compared to cells or animals without such alleles. The degree of elevation of the spontaneous mutation rate can be at least 2-
20 fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 500-fold, or 1000-fold that of the normal cell or animal. The use of chemical mutagens such as but limited to methane sulfonate, dimethyl sulfonate, O6-methyl benzadine, MNU, ENU, etc. can be used in MMR defective cells to increase the rates an additional 10 to 100 fold that of the MMR deficiency itself.

25 According to one aspect of the invention, a polynucleotide encoding for a dominant negative form of a MMR protein is introduced into a cell. The gene can be any dominant negative allele encoding a protein, which is part of a MMR complex, for example, *PMS2*, *PMS1*, *MLH1*, or *MSH2*. The dominant negative allele can be naturally occurring or made in the laboratory. The polynucleotide can be in the form of genomic DNA, cDNA, RNA,
30 or a chemically synthesized polynucleotide.

The polynucleotide can be cloned into an expression vector containing a constitutively active promoter segment (such as but not limited to CMV, SV40, Elongation Factor or LTR sequences) or to inducible promoter sequences such as the steroid inducible

pIND vector (Invitrogen), where the expression of the dominant negative MMR gene can be regulated. The polynucleotide can be introduced into the cell by transfection.

According to another aspect of the invention, an immunoglobulin (Ig) gene, a set of Ig genes or a chimeric gene containing whole or parts of an Ig gene can be transfected into
5 MMR deficient cell hosts, the cell is grown and screened for clones containing genetically altered Ig genes with new biochemical features. MMR defective cells may be of human, primates, mammals, rodent, plant, yeast or of the prokaryotic kingdom. The mutated gene encoding the Ig with new biochemical features may be isolated from the respective clones and introduced into genetically stable cells (*i.e.*, cells with normal MMR) to provide clones
10 that consistently produce Ig with the new biochemical features. The method of isolating the Ig gene encoding Ig with new biochemical features may be any method known in the art. Introduction of the isolated polynucleotide encoding the Ig with new biochemical features may also be performed using any method known in the art, including, but not limited to transfection of an expression vector containing the polynucleotide encoding the
15 Ig with new biochemical features. As an alternative to transfecting an Ig gene, a set of Ig genes or a chimeric gene containing whole or parts of an Ig gene into an MMR deficient host cell, such Ig genes may be transfected simultaneously with a gene encoding a dominant negative mismatch repair gene into a genetically stable cell to render the cell hypermutable.

20 Transfection is any process whereby a polynucleotide is introduced into a cell. The process of transfection can be carried out in a living animal, *e.g.*, using a vector for gene therapy, or it can be carried out *in vitro*, *e.g.*, using a suspension of one or more isolated cells in culture. The cell can be any type of eukaryotic cell, including, for example, cells isolated from humans or other primates, mammals or other vertebrates, invertebrates, and
25 single celled organisms such as protozoa, yeast, or bacteria.

In general, transfection will be carried out using a suspension of cells, or a single cell, but other methods can also be applied as long as a sufficient fraction of the treated cells or tissue incorporates the polynucleotide so as to allow transfected cells to be grown and utilized. The protein product of the polynucleotide may be transiently or stably
30 expressed in the cell. Techniques for transfection are well known. Available techniques for introducing polynucleotides include but are not limited to electroporation, transduction, cell fusion, the use of calcium chloride, and packaging of the polynucleotide together with lipid for fusion with the cells of interest. Once a cell has been transfected with the MMR

gene, the cell can be grown and reproduced in culture. If the transfection is stable, such that the gene is expressed at a consistent level for many cell generations, then a cell line results.

An isolated cell is a cell obtained from a tissue of humans or animals by
5 mechanically separating out individual cells and transferring them to a suitable cell culture medium, either with or without pretreatment of the tissue with enzymes, *e.g.*, collagenase or trypsin. Such isolated cells are typically cultured in the absence of other types of cells. Cells selected for the introduction of a dominant negative allele of a mismatch repair gene may be derived from a eukaryotic organism in the form of a primary cell culture or an
10 immortalized cell line, or may be derived from suspensions of single-celled organisms.

A polynucleotide encoding for a dominant negative form of a MMR protein can be introduced into the genome of an animal by producing a transgenic animal. The animal can be any species for which suitable techniques are available to produce transgenic animals. For example, transgenic animals can be prepared from domestic livestock, *e.g.*,
15 bovine, swine, sheep, goats, horses, etc.; from animals used for the production of recombinant proteins, *e.g.*, bovine, swine, or goats that express a recombinant polypeptide in their milk; or experimental animals for research or product testing, *e.g.*, mice, rats, guinea pigs, hamsters, rabbits, etc. Cell lines that are determined to be MMR defective can then be used as a source for producing genetically altered immunoglobulin genes *in vitro*
20 by introducing whole, intact immunoglobulin genes and/or chimeric genes encoding for single chain antibodies into MMR defective cells from any tissue of the MMR defective animal.

Once a transfected cell line or a colony of transgenic animals has been produced, it can be used to generate new mutations in one or more gene(s) of interest. A gene of
25 interest can be any gene naturally possessed by the cell line or transgenic animal or introduced into the cell line or transgenic animal. An advantage of using such cells or animals to induce mutations is that the cell or animal need not be exposed to mutagenic chemicals or radiation, which may have secondary harmful effects, both on the object of the exposure and on the workers. However, chemical mutagens may be used in
30 combination with MMR deficiency, which renders such mutagens less toxic due to an undetermined mechanism. Hypermutable animals can then be bred and selected for those producing genetically variable B-cells that may be isolated and cloned to identify new cell lines that are useful for producing genetically variable cells. Once a new trait is identified,

the dominant negative MMR gene allele can be removed by directly knocking out the allele by technologies used by those skilled in the art or by breeding to mates lacking the dominant negative allele to select for offspring with a desired trait and a stable genome.

Another alternative is to use a CRE-LOX expression system, whereby the dominant

- 5 negative allele is spliced from the animal genome once an animal containing a genetically diverse immunoglobulin profile has been established. Yet another alternative is the use of inducible vectors such as the steroid induced pIND (Invitrogen) or pMAM (Clonotech) vectors which express exogenous genes in the presence of corticosteroids.

- Mutations can be detected by analyzing for alterations in the genotype of the cells
10 or animals, for example by examining the sequence of genomic DNA, cDNA, messenger RNA, or amino acids associated with the gene of interest. Mutations can also be detected by screening for the production of antibody titers. A mutant polypeptide can be detected by identifying alterations in electrophoretic mobility, spectroscopic properties, or other physical or structural characteristics of a protein encoded by a mutant gene. One can also
15 screen for altered function of the protein *in situ*, in isolated form, or in model systems. One can screen for alteration of any property of the cell or animal associated with the function of the gene of interest, such as but not limited to Ig secretion.

Examples of mismatch repair proteins and nucleic acid sequences include the following:

20

PMS2 (mouse) (SEQ ID NO:5)

- | | | | | | | | |
|----|------------|------------|-------------|------------|------------|------------|-----|
| | MEQTEGVSTE | CAKAIKPIDG | KSVHQICSGQ | VILSLSTAVK | ELIENSVDAG | ATTIDLRLKD | 60 |
| | YGVDLIEVSD | NGCGVEEENF | EGLALKHHTS | KIQEFADLTQ | VETFGFRGEA | LSSLCALSDV | 120 |
| | TISTCHGSAS | VGTRLVFDHN | GKITQKTPYP | RPKGTTVSQ | HLFYTLPVRY | KEFQRNIKKE | 180 |
| 25 | YSKMQVQLQA | YCIISAGVRV | SCTNQLGQGK | RHAVVCTSGT | SGMKENIGSV | FGQKQLQSLI | 240 |
| | PFVQLPPSDA | VCEEYGLSTS | GRHKTFTFTR | ASFHSARTAP | GGVQQTGSFS | SSIRGPVTQQ | 300 |
| | RSLSLSMRFY | HMYNRHQYPF | VVLNVSVSDSE | CVDINVTDPK | RQILLQEEKL | LLAVLKTSLI | 360 |
| | GMFDSANKL | NVNQQPLLDV | EGNLVKLHTA | ELEKPVPGKQ | DNSPSLKSTA | DEKRVASISR | 420 |
| | LREAFSLHPT | KEIKSRGPET | AELTRSPSE | KRGVLSSYPS | DVISYRGLRG | SQDKLVSPTD | 480 |
| 30 | SPGDCMDREK | IEKDSGLSST | SAGSEEEFST | FEVASSFSSD | YNVSSLEDRL | SQETINCGDL | 540 |
| | DCRPPGTGQS | LKPEDHGYQC | KALPLARLSP | TNAKRFKTEE | RPSNVNISQR | LPGPQSTSAA | 600 |
| | EVDVAIKMNK | RIVLLEFSL | SLAKRMKQLQ | HLKAQNKHEL | SYRKFRKIC | PGENQAAEDE | 660 |
| | LRKEISKSMF | AEMEILGQFN | LGFIIVTKLKE | DLFLVDQHAA | DEKYNFEMLQ | QHTVLQAQRL | 720 |
| | ITPQTLNLTA | VNEAVLIENL | EIFRKNGFDF | VIDEDAPVTE | RAKLISLPTS | KNWTFGPQDI | 780 |
| 35 | DELIFMLSDS | PGVMCRPSRV | QMFASRACR | KSVMIGTALN | ASEMKKLITH | MGEMDHPWNC | 840 |
| | PHGRPTMRHV | ANLDVISQN | | | | | 859 |

PMS2 (mouse cDNA) (SEQ ID NO:6)

- | | | | | | | | |
|----|------------|------------|------------|------------|------------|------------|-----|
| 40 | gaattccggt | gaaggtcctg | aagaatttcc | agattcctga | gtatcattgg | aggagacaga | 60 |
| | taacctgtcg | tcaggtaacg | atggtgtata | tgcaacagaa | atgggtgttc | ctggagacgc | 120 |
| | gtcttttccc | gagagcggca | cgcgaactct | ccgcggtga | ctgtgactgg | aggagtcctg | 180 |
| | catccatgga | gcaaaccgaa | ggcgtgagta | cagaatgtgc | taaggccatc | aagcctattg | 240 |
| | atgggaagtc | agtccatcaa | atttgttctg | ggcaggtgat | actcagttta | agcaccgctg | 300 |
| 45 | tgaaggagtt | gatagaaaat | agtgtagatg | ctggtgctac | tactattgat | ctaaggctta | 360 |

	aagactatgg	ggtggacctc	attgaagttt	cagacaatgg	atgtggggta	gaagaagaaa	420
	actttgaagg	tctagctctg	aaacatcaca	catctaagat	tcaagagttt	gccgacctca	480
	cgcagggtga	aactttcggc	tttcgggggg	aagctctgag	ctctctgtgt	gcactaagtg	540
5	atgtcactat	atctacctgc	cacgggtctg	caagcggttg	gactcgactg	gtgtttgacc	600
	ataatgggaa	aatcaccag	aaaactccct	acccccgacc	taaaggaacc	acagtcagtg	660
	tgcagcactt	attttataca	ctacccgtgc	gttacaaga	gtttcagagg	aacattaaaa	720
	aggagtattc	caaaatggtg	caggtcttac	aggcgtagtg	tatcatctca	gcaggcggtcc	780
	gtgtaagctg	cactaatcag	ctcggacagg	ggaagcggca	cgctgtggtg	tgcacaagcg	840
10	gcacgtctgg	catgaaggaa	aatatcgggt	ctgtgttttg	ccagaagcag	ttgcaaagcc	900
	tcattccttt	tgttcagctg	ccccctagtg	acgctgtgtg	tgaagagtac	ggcctgagca	960
	cttcaggacg	ccacaaaacc	ttttctacgt	ttcgggcttc	atttcacagt	gcacgcacgg	1020
	cgcggggagg	agtgcacag	acaggcagtt	tttcttcac	aatcagaggc	cctgtgacct	1080
	agcaaaagtc	tctaagcttg	tcaatgaggt	tttatcacat	gtataaccgg	catcagtagc	1140
	catttgtcgt	ccttaacggt	tccgttgact	cagaatgtgt	ggatattaat	gtaactccag	1200
15	ataaaaggca	aattctacta	caagaagaga	agctattgct	ggcggtttta	aagacctcct	1260
	tgataggaat	gtttgacagt	gatgcaaa	agcttaagt	caaccagcag	ccactgctag	1320
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	agcaagataa	ctctccttca	ctgaagagca	cagcagacga	gaaaagggtg	gcatccatct	1440
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	agactgctga	actgacacgg	agttttccaa	gtgagaaaag	ggcgctgtta	tcctcttacc	1560
	cttcagacgt	catctcttac	agaggcctcc	gtggctcgca	ggacaaattg	gtgagtccca	1620
	cggacagccc	tggtgactgt	atggacagag	agaaaataga	aaaagactca	gggctcagca	1680
	gcacctcagc	tggtctctgag	gaagagttca	gcaccccgaga	agtggccagt	agcttttagca	1740
25	gtgactataa	cgtgagctcc	ctagaagaca	gaccttctca	ggaaaccata	aactgtgggtg	1800
	acttggtact	ccgtcctcca	ggtacaggac	agtccctgaa	gccagaagac	catggatatt	1860
	aatgcaaagc	tctacctcta	gctcgtctgt	caccacaaaa	tgccaagcgc	ttcaagacag	1920
	aggaaagacc	ctcaaagtgc	aacattttct	aaagattgcc	tggtcctcag	agcacctcag	1980
	cagctgaggt	cgatgtagcc	ataaaaaatga	ataagagaat	cgtgctcctc	gagttctctc	2040
30	tgagttctct	agctaagcga	atgaagcagt	tacagcacct	aaaggcgcag	aacaaacatg	2100
	aactgagttt	cagaaaattt	agggccaa	tttgccctgg	agaaaaccaa	gcagcagaag	2160
	atgaactcag	aaaagagatt	agtaaatcga	tggttgacga	gatggagatc	ttgggtcagt	2220
	ttaacctggg	atttatagta	accaaaactga	aagaggacct	cttccctggg	gaccagcatg	2280
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	ctgaaagggc	taaattgatt	tccttaccac	ctagtaaaaa	ctggaccttt	ggaccccaag	2520
	atatagatga	actgatcttt	atgttaagtg	acagccctgg	ggtcatgtgc	cggccctcac	2580
	gagtcagaca	gatgtttgct	tccagagcct	gtcgaagtc	agtgatgatt	ggaacggcgc	2640
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	actgccccca	cggcaggcca	accatgaggc	acgttgccaa	tctggatgtc	atctctcaga	2760
	actgacacac	cccttgtagc	atagagttta	ttacagattg	ttcgggtttgc	aaagagaagg	2820
	ttttaagtaa	tctgattatc	gttgtaaaaa	aattagcatg	ctgctttaat	gtactggatc	2880
	catttaaaag	cagtgttaag	gcaggcatga	tggagtgttc	ctctagctca	gctacttggg	2940
45	tgatccgggt	ggagctcatg	tgagccagc	actttgagac	cactccgagc	cacattcatg	3000
	agactcaatt	caaggacaaa	aaaaaaaaaga	tatttttgaa	gccttttaaa	aaaaaa	3056

PMS2 (human) (SEQ ID NO:7)

	MERAESSSTE	PAKAIKPIDR	KSVHQICSGQ	VVLSLSTAVK	ELVENS LDAG	ATNIDLK LKD	60
	YGVDLIEVSD	NGCGVEEENF	EGLTLKHHTS	KIQEFADLTQ	VETFGFRGEA	LSSL CALSDV	120
50	TISTCHASAK	VGTRLMFDHN	GKIIQKTPYP	RPRGTTVSQ	QLFSTLPVRH	KEFQRNIKE	180
	YAKMVQVLHA	YCIISAGIRV	SCTNQLGQK	RQPVVCTGGS	PSIKENIGSV	FGQKQLQSLI	240
	PFVQLPPSDS	VCEEYGLSCS	DALHNLFYIS	GFISQCTHGV	GRSSTDROFF	FINRRPCDPA	300
	KVCRLVNEVY	HMYNRHQYPF	VVLNISVDSE	CVDINVTDPK	RQILLQEEKL	LLAVLKTSLI	360
	GMFDSVDNKL	NVSQQPLLDV	EGNLIKMHAA	DLEKPMVEKQ	DQSPSLRTGE	EKKDVSISRL	420
55	REAFSLRHHT	ENKPHSPKTP	EPRRSPLGQK	RGMLSSSTSG	AISDKGVLRP	QKEAVSSSHG	480
	PSDPTDRAEV	EKDSGHGSTS	VDSEGF SIPD	TGSHCSSEYA	ASSPGDRGSQ	EHVDSQEKAP	540
	ETDDSFSDVD	CHSNQEDTGC	KFRVLPQPTN	LATPNTKRFK	KEEILSSSDI	CQKL VNTQDM	600
	SASQVDVAVK	INKKVPLDF	SMSSLAKRIK	QLHHEAQQSE	GEQNYRK FRA	KICPGENQAA	660
	EDEL RKEISK	TMFAEMEIIIG	QFNLFGIITK	LNEDIFIVDQ	HATDEKYNFE	MLQQHTVLQG	720
60	QRLIAPQTLN	LTAVNEAVLI	ENLEIFRKNG	FDFVIDENAP	VTERAKLISL	PTSKNWTFGP	780
	QVDDELIFML	SDSPGVMC RP	SRVKQMFASR	ACRKSVMIGT	ALNTSEMKKL	ITHMGEMDHP	840
	WNCPHGRPTM	RHIANLGVIS	QN				862

PMS2 (human cDNA) (SEQ ID NO:8)

	cgaggcggat	cgggtgttgc	atccatggag	cgagctgaga	gctcaggtac	agaacctgct	60
	aaggccatca	aacctattga	tcggaagtca	gtccatcaga	tttgctctgg	gcaggtggtg	120
	ctgagtctaa	gcactgcggt	aaaggagtta	gtagaaaaca	gtctggatgc	tggtgccact	180
5	aatattgatc	taaagcttaa	ggactatgga	gtggatctta	ttgaagtttc	agacaatgga	240
	tgtggggtag	aagaagaaaa	cttcgaaggc	ttaactctga	aacatcacac	atctaagatt	300
	caagagtttg	ccgacctaac	tcaggttgaa	actttttggt	ttcgggggga	agctctgagc	360
	tcacttttgt	cactgagcga	tgtcaccatt	tctacctgcc	acgcatcgcc	gaaggttggg	420
	actcgactga	tgtttgatca	caatgggaaa	attatccaga	aaacccctta	ccccgcccc	480
10	agagggacca	cagtcagcgt	gcagcagtta	ttttccacac	tacctgtgcg	ccataaggaa	540
	tttcaaagga	atattaagaa	ggagtatgcc	aaaatggtcc	aggtcttaca	tgcatactgt	600
	atcattttcag	caggcatccg	tgtaagttgc	accaatcagc	ttggacaagg	aaaacgcagc	660
	cctgtgttat	gcacaggtgg	aagccccagc	ataaaggaaa	atatcggtc	tgtgtttggg	720
	cagaagcagt	tgcaaaagcct	cattcctttt	gttcagctgc	cccctagtga	ctccgtgtgt	780
15	gaagagtacg	gtttgagctg	ttcggatgct	ctgcataatc	ttttttacat	ctcaggtttc	840
	atttcacaat	gcacgcagtg	agttggaagg	agttcaacag	acagacagtt	tttctttatc	900
	aaccggcgcc	cttgtgaccc	agcaaaggtc	tcgagactcg	tgaatgaggt	ctaccacatg	960
	tataatcgac	accagtatcc	atttggtgtt	cttaacattt	ctggtgattc	agaatgcgtt	1020
	gatatcaatg	ttactccaga	taaaaggcaa	attttgctac	aagaggaaaa	gcttttgttg	1080
20	gcagtttttaa	agacctcttt	gataggaatg	tttgatagtg	atgtcaacaa	gctaaatgtc	1140
	agtcagcagc	cactgctgga	tggtgaaggt	aacttaataa	aatgcatgc	agcggatttg	1200
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	aagcctcaca	gcccacagac	tccagaacca	agaaggagcc	ctctaggaca	gaaaaggggt	1380
25	atgctgtctt	ctagcacttc	aggtgccatc	tctgacaaa	gcgtcctgag	acctcagaaa	1440
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	agtcactgca	gcagcgagta	tgccggccagc	tccccagggg	acaggggctc	gcaggaacat	1620
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30	tcaaaccagg	aagataccgg	atgtaaattt	cgagttttgc	ctcagccaac	taatctcgca	1740
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	aagtttagtaa	atactcagga	catgtcagcc	tctcaggttg	atgtagctgt	gaaaattaat	1860
	aagaaagttg	tgccccctgga	cttttctatg	agttcttttag	ctaaacgaat	aaagcagtta	1920
	catcatgaag	caagcaaaag	tgaaggggaa	cagaattaca	ggaagtttag	ggcaaagatt	1980
35	tgtcctggag	aaaatcaagc	agccgaagat	gaactaagaa	aagagataag	taaaacgatg	2040
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	gaggatatct	tcatagtgga	ccagcatgcc	acggacgaga	agtataaact	cgagatgctg	2160
	cagcagcaca	ccgtgctcca	ggggcagagg	ctcatagcac	ctcagactct	caacttaact	2220
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40	tttgttatcg	atgaaaatgc	tccagtcact	gaaagggcta	aactgatttc	cttgccaact	2340
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	agccctgggg	tcattgtccg	gccttcccga	gtcaagcaga	tggttgccctc	cagagccctgc	2460
	cggaagtcgg	tgatgattgg	gactgctctt	aacacaagcg	agatgaagaa	actgatcacc	2520
	cacatggggg	agatggacca	cccctggaac	tgcccccatg	gaaggccaac	catgagacac	2580
45	atcgccaacc	tgggtgtcat	ttctcagaac	tgaccgtagt	cactgtatgg	aataattggg	2640
	tttatcgag	atttttatgt	tttgaagac	agagtcttca	ctaacccttt	ttgttttaaa	2700
	atgaaacctg	ctacttaaaa	aaaatacaca	tcacacccat	ttaaaagtga	tcttgagaac	2760
	cttttcaaac	c					2771

50 PMS1 (human) (SEQ ID NO:9)

	MKQLPAATVR	LLSSSQIITS	VVSUVKELIE	NSLDAGATSV	DVKLENYGFD	KIEVRDNGEG	60
	IKAVDAPVMA	MKYYSKINS	HEDLENLTYY	GFRGEALGSI	CCIAEVLITT	RTAADNFSTQ	120
	YVLDGSGHIL	SQKPSHLGQG	TTVTALRLFK	NLPVRKQFYS	TAKKCKDEIK	KIQDLLMSFG	180
	ILKPDRLRIVF	VHNKAVIWQK	SRVSDHKMAL	MSVLGTAVMN	NMESFQYHSE	ESQIYLSGFL	240
55	PKCDADHSFT	SLSTPERSFI	FINSRPVHQK	DILKLIRHHY	NLKLKESTR	LYPVFFLKID	300
	VPTADVVDNL	TPDKSQVLLQ	NKESVLIALE	NLMTTCYGPL	PSTNSYENNK	TDVSAADIVL	360
	SKTAETDVLF	NKVESGKNY	SNVDTSVIPF	QNDMHNDESG	KNTDDCLNHQ	ISIGDFGYGH	420
	CSSEISNIDK	NTKNAFQDIS	MSNVSWENSQ	TEYSKTCFIS	SVKHTQSENG	NKDHIDESGE	480
	NEEEAGLENS	SEISADEWSR	GNILKNSVGE	NIEPVKILVP	EKSLPCKVSN	NNYPIPEQMN	540
60	LNEDSCNKK	NVIDNKGSKV	TAYDLLSNR	IKKPMASAL	FVQDHRPQFL	IENPKTSLED	600
	ATLQIEELWK	TLSEEEKLKY	EEKATKDRL	YNSQMKRAIE	QESQMSLKDG	RKKIKPTSAA	660
	NLAQKHKLKT	SLSNQPKLDE	LLQSQIEKRR	SQNIKMVQIP	FSMKNLKINF	KKQNKVDLEE	720
	KDEPCLIHNL	RFPDAWLMTS	KTEVMLLPY	RVEEALLFKR	LLENHKLPAE	PLEKPIMLTE	780
	SLFNGSHYLD	VLYKMTADDQ	RYSGSTYLS	PRLTANGFKI	KLIPGVSITE	NYLEIEGMAN	840
65	CLPFYGVADL	KEILNAILNR	NAKEVYECRP	RKVISYLEGE	AVRLSRQLPM	YLSKEDIQDI	900
	IYRMKHQFGN	EIKECVHGRP	FFHHLTYPLE	TT			932

PMS1 (human) (SEQ ID NO:10)

	ggcacgagtg	gctgcttgcg	gctagtggat	ggtaattgcc	tgccctgcgc	tagcagcaag	60
5	ctgctctgtt	aaaagcgaaa	atgaaacaat	tgccctgcgc	aacagttcga	ctcctttcaa	120
	gttctcagat	catcacttcg	gtggtcagtg	ttgtaaaaga	gcttattgaa	aactccttgg	180
	atgctgggtg	cacaagcgta	gatgttaaac	tgagagaacta	tggaattgat	aaaattgagg	240
	tgcgagataa	cggggagggt	atcaaggctg	ttgatgcacc	tgtaattggca	atgaagtact	300
	acacctcaaa	aataaatagt	catgaagatc	ttgaaaattt	gacaacttac	ggttttcgtg	360
10	gagaagcctt	gggttcaatt	tggtgtatag	ctgaggtttt	aattacaaca	agaacggctg	420
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	atctcctcat	gagctttgg	atccttaaac	ctgacttaag	gattgtcttt	gtacataaca	660
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15	tggggactgc	tggtatgaac	aatatggaat	cctttcagta	ccactctgaa	gaatctcaga	780
	tttatctcag	tggttttctt	ccaaagtgtg	atgcagacca	ctctttcact	agtctttcaa	840
	caccagaaa	aagtttcatc	ttcataaaaa	gtcgaccagt	acatcaaaaa	gatattctta	900
	agttaatccg	acatcattac	aatctgaaat	gcctaaagga	atctactcgt	ttgtatcctg	960
	ttttctttct	gaaaatcgat	gttcctacag	ctgatgttga	tgtaaatatta	acaccagata	1020
20	aaagccaagt	attattacaa	aataaggaat	ctgttttaag	tgctcttgaa	aatctgatga	1080
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35	aagaggaaaa	actgaaatat	gaagagaagg	ctactaaaga	cttggaacga	tacaatagtc	1980
	aaatgaagag	agccattgaa	caggagtcac	aaatgtcact	aaaagatggc	agaaaaaaga	2040
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	aagccctgct	atttaaaaga	cttcttgaga	atcataaact	tcctgcagag	ccactggaaa	2400
	agccaattat	gttaacagag	agtcttttta	atggatctca	ttatttagac	gttttatata	2460
	aatgacacg	agatgaccaa	agatacagtg	gatcaactta	cctgtctgat	cctcgtctta	2520
45	cagcgaatgg	tttcaagata	aaattgatac	caggagtttc	aattactgaa	aattacttgg	2580
	aaatagaagg	aatggctaat	tgtctcccat	tctatggagt	agcagattta	aaagaaattc	2640
	ttaatgctat	attaaacaga	aatgcaaagg	aagtttatga	atgtagacct	cgcaaagtga	2700
	taagtatttt	agagggagaa	gcagtgcgtc	tatccagaca	attacccatg	tacttatcaa	2760
	aagaggacat	ccaagacatt	atctacagaa	tgaagcacca	gtttggaaat	gaaattaaag	2820
50	agtgtgttca	tggtcgccca	ttttttcatc	atttaaccta	tcttcagaaa	actacatgat	2880
	taaatatgtt	taagaagatt	agttaccatt	gaaattgggt	ctgtcataaa	acagcatgag	2940
	tctgtgttta	aattatcttt	gtattatgtg	tcacatgggt	attttttaaa	tgaggattca	3000
	ctgacttggt	tttatattga	aaaaagttcc	acgtattgta	gaaaacgtaa	ataaactaat	3060
55	aac						3063

MSH2 (human) (SEQ ID NO:11)

60	MAVQPKETLQ	LESAAEVGFV	RFFQGMPEKP	TTTVRLFDRG	DFYTAHGEDA	LLAAREVFKT	60
	QGVIKYMGPA	GAKNLQSVVL	SKMNFESFVK	DLILVRQYRV	EVYKNRAGNK	ASKENDWYLA	120
	YKASPGNLSQ	FEDILFGNND	MSASIGVVG	KMSAVDQGRQ	VGVGVDISIQ	RKLGLCEFPD	180
	NDQFSLNEAL	LIQIGPKCEV	LPGGETAGDM	GKLRQIIQRQ	GILITERKKA	DFSTKDIYQD	240
	LNRLKGGKKG	EQMNSAVLPE	MENQVAVSSL	SAVIKLELL	SDDSNFGQFE	LTTDFDSQYM	300
65	KLDIAAVRAL	NLFQGSVEDT	TGSQSLAALL	NKCKTPQGQR	LVNQWIKQPL	MDKNRIEERL	360

	NLVEAFVEDA	ELRQTLQEDL	LRRFPDLNRL	AKKFQROAAN	LQDCYRLYQG	INQLPNVIAQ	420
	LEKHEGKHQK	LLLAFFVTPL	TDLRSDFSKF	QEMIETTLDM	DQVENHEFLV	KPSFDPNLSE	480
	LREIMNDLEK	KMQSTLISAA	RDLGLDPGKQ	IKLDSSAQFG	YYFRVTCKEE	KVLRRNNKNFS	540
	TVDIQKNGVK	FTNSKLTSLN	BEYTKNKTEY	EEAQDAIVKE	IVNISSGYVE	PMQTLNDVLA	600
5	QLDAVVSFAH	VSNGAPVPYV	RPAILEKGQG	RIILKASRHA	CVEVQDEIAF	IPNDVYFEKD	660
	KQMFHIITGP	NMGGKSTYIR	QTGVIVLMAQ	IGCFVPCESA	EVSIVDCILA	RVGAGDSQLK	720
	GVSTFMAEML	ETASILRSAT	KDSLIIIDEL	GRGTSTYDGF	GLAWAISEYI	ATKIGAFCMF	780
	ATHFHELTAL	ANQIPTVNNL	HVTALTTEET	LTMLYQVKKG	VCDQSFQIHV	AELANFPKHV	840
	IECAKQKALE	LEEFQYIGES	QGYDIMEPAA	KKCYLEREQG	EKIIQEFLSK	VKQMPFTEMS	900
10	EENITIKLKQ	LKAEVIAKNN	SFVNEIISRI	KVTT			934

MSH2 (human cDNA) (SEQ ID NO:12)

	ggcgggaaac	agcttagtg	gtgtgggggtc	gogcattttc	ttcaaccagg	aggtgaggag	60
	gtttcgacat	ggcgggtgcag	ccgaaggaga	cgctgcagtt	ggagagcgcg	gcccgaggtcg	120
15	gcttcgtgcg	cttctttcag	ggcatgccgg	agaagccgac	caccacagtg	cgcccttttcg	180
	accggggcga	cttctatacg	gcgcacggcg	aggacgcgct	gctggccgcc	cgggaggtgt	240
	tcaagaccca	gggggtgatc	aagtacatgg	ggccggcagg	agcaaaagaa	ctgcagagtg	300
	ttgtgcttag	taaaatgaat	tttgaatctt	ttgtaaaaga	tcttcttctg	gttcgtcagt	360
	atagatttga	agtttataag	aatagagctg	gaaataaggc	atccaaggag	aatgattggt	420
20	atattggcata	taaggcttct	cctggcaatc	tctctcagtt	tgaagacatt	ctctttgtga	480
	acaatgatat	gtcagcttcc	attgggtgtt	tgggtgttaa	aatgtccgca	gttgatggcc	540
	agagacaggt	tggagttggg	tatgtggatt	ccatacagag	gaaactagga	ctgtgtgaat	600
	tccctgataa	tgatcagttc	tccaatcttg	aggctctcct	catccagatt	ggaccaaaag	660
	aatgtgtttt	accgggagga	gagactgctg	gagacatggg	gaaactgaga	cagataattc	720
25	aaagaggagg	aattctgatc	acagaaaaga	aaaaagctga	cttttccaca	aaagacattt	780
	atcaggacct	caaccggttg	ttgaaaggca	aaaagggaga	gcagatgaat	agtgtgtgat	840
	tgccagaaat	ggagaatcag	gttgacgttt	catcactgtc	tgcggtaatc	aagtttttag	900
	aactcttata	agatgattcc	aactttggac	agtttgaact	gactactttt	gacttcagcc	960
	agtatatgaa	attggatatt	gcagcagtc	gagcccttaa	cctttttcag	ggttctgttg	1020
30	aagataccac	tggtctctcag	tctctggctg	ccttgctgaa	taagtgtaaa	acccctcaag	1080
	gacaaagact	tgtaaccag	tggattaagc	agcctctcat	ggataagaac	agaatagagg	1140
	agagattgaa	tttagtgga	gctttttag	aagatgcaga	attgaggcag	actttacaag	1200
	aagatttact	ctgtcgattc	ccagatctta	accgacttgc	caagaagttt	caaagacaag	1260
	cagcaaaact	acaagattgt	taccgactct	atcagggtat	aaatcaacta	cctaattgta	1320
35	tacaggctct	ggaaaaacat	gaaggaaaac	accagaaatt	attgttggca	gtttttgtga	1380
	ctcctcttac	tgatcttcgt	tctgacttct	ccaagtttca	ggaaatgata	gaaacaactt	1440
	tagatatgga	tcagggtgga	aaccatgaat	tccttgtaaa	accttcattt	gattcctaac	1500
	tcagtgaatt	aagagaaata	atgaatgact	tggaaaagaa	gatgcagtc	acattaataa	1560
	gtgcagccag	agatcttggc	ttggaccctg	tggaacagat	taaaactggat	tccagtgcac	1620
40	agtttggata	ttactttcgt	gtaacctgta	aggaagaaaa	agtccttcgt	aacaataaaa	1680
	acttttagtac	tgtagatata	cagaagaatg	gtgttaaaat	taccaacagc	aaattgactt	1740
	ctttaaatga	agagtatacc	aaaaataaaa	cagaatatga	agaagcccag	gatgccattg	1800
	ttaaagaatt	tgtcaatatt	tcttcaggct	atgtagaacc	aatgcagaca	ctcaatgatg	1860
	tgtagactca	gctagatgct	gttgtcagct	ttgtcacgt	gtcaaatgga	gcacctgttc	1920
45	catatgtacg	accagccatt	ttggagaaag	gacaaggaag	aattatatta	aaagcatcca	1980
	ggcatgcttg	tggtgaagtt	caagatgaaa	ttgcatttat	tcctaataac	gtatactttg	2040
	aaaaagataa	acagatgttc	cacatcatta	ctggccccaa	tatgggaggt	aaatcaacat	2100
	atattcgaca	aactgggggtg	atagtaactc	tggcccaaat	tgggtgtttt	gtgccatgtg	2160
	agtcagcaga	agtgctccat	gtggactgca	tcttagcccg	agtaggggct	ggtgacagtc	2220
50	aattgaaagg	agtcctccag	tccatggctg	aaatgttgga	aactgcttct	atcctcaggt	2280
	ctgcaaccaa	agattcatta	ataatcatag	atgaattggg	aagaggaact	tctacctacg	2340
	atggattttg	gttagcatgg	gctatatcag	aatacattgc	aacaaagatt	ggtgcttttt	2400
	gcattgtttg	aacccatttt	catgaactta	ctgccttggc	caatcagata	ccaactgtta	2460
	ataatctaca	tgtaacagca	ctcaccactg	aagagacctt	aactatgctt	tatcaggtga	2520
55	agaaaggtgt	ctgtgatcaa	agttttggga	tccatgttgc	agagcttgct	aatttcccta	2580
	agcatgtaat	agagtgtgct	aaacagaaag	ccctggaact	tgaggagttt	cagtatattg	2640
	gagaatcgca	aggatatgat	atcatggaac	cagcagcaaa	gaagtgtctat	ctggaaaagag	2700
	agcaaggtga	aaaaattatt	caggagttcc	tgtccaaggt	gaaacaaatg	ccctttactg	2760
	aaatgtcaga	agaaaacatc	acaataaagt	taaaacagct	aaaagctgaa	gtaatagcaa	2820
60	agaataatag	ctttgtaaat	gaaatcattt	cacgaataaa	agttactaag	tgaaaaatcc	2880
	cagtaattgga	atgaaggtaa	tattgataag	ctattgtctg	taatagtgtt	atatgttttt	2940
	atattaaccc	tttttccata	gtgttaactg	ttagtgccca	tgggctatca	acttaataag	3000
	atatttagta	atattttact	ttgaggacat	tttcaaagat	ttttattttg	aaaaatgaga	3060
	gctgtaactg	aggactgttt	gcaattgaca	taggcaataa	taagtgtgtg	gctgaatttt	3120
65	ataaataaaa	tcatgtagtt	tgtgg				3145

MLH1 (human) (SEQ ID NO:13)

	MSFVAGVIRR	LDETVVNRIA	AGEVIQRPAN	AIKEMIENCL	DAKSTSIQVI	VKEGGLKLIQ	60
	IQDNGTGIRK	EDLDIVCERF	TTSKLQSFED	LASISTYGFR	GEALASISHV	AHVTITTTKA	120
5	DGKCAYRASY	SDGKLKAPPK	PCAGNQGTQI	TVEDLFYNIA	TRRKALKNPS	EEYGKILEVV	180
	GRYSVHNAGI	SFSVKKQGET	VADVRLPNA	STVDNIRSIF	GNAVSRELIE	IGCEDKTLAF	240
	KMNGYISNAN	YSVKKCIFLL	FINHRLVEST	SLRKAJETVY	AAYLPKNTHP	FLYLSLEISP	300
	QNVVDNVHPT	KHEVHFLHEE	SILERVQQHI	ESKLLGSNSS	RMFTQTLLP	GLAGPSGEMV	360
	KSTTSLTSSS	TSGSSDKVYA	HQMVRTDSRE	QKLD AFLQPL	SKPLSSQPQA	IVTEDKTDIS	420
10	SGRARQQDEE	MLELPAPAEV	AAKNQSLEGD	TTKGTSEMSE	KRGPTSSNPR	KRHREDS DVE	480
	MVEDDSRKEM	TAACTPRRRI	INLTSVLSLQ	EEINEQGHEV	LREMLHNHSF	VGCVNPQWAL	540
	AQHQTCLYLL	NTTKLSEELF	YQILIYDFAN	FGVLR LSEPA	PLFDLAMLAL	DSPESGWTEE	600
	DGPKEGLAEY	IVEFLKKKAE	MLADYFSLEI	DEEGNLIGLP	LLIDNYVPPL	EGLPIFILRL	660
	ATEVNWDEEK	ECFESLSKEC	AMFYSIRKQY	ISEESTLSGQ	QSEVPGSIPN	SWKWTVEHIV	720
15	YKALRSHILP	PKHFTEDGNI	LQLANLPDLY	KVFERC			756

MLH1 (human) (SEQ ID NO:14)

	cttggtctctt	ctggcgccaa	aatgtcgcttc	gtggcagggg	ttattcggcg	gctggacgag	60
	acagtgggtga	accgcatcgc	ggcgggggaa	gttatccagc	ggccagctaa	tgctatcaaa	120
20	gagatgattg	agaactgttt	agatgcaaaa	tccacaagta	ttcaagtgat	tgtaaagag	180
	ggaggcctga	agttgattca	gatccaagac	aatggcaccg	ggatcaggaa	agaagatctg	240
	gatatgtgat	gtgaaagggt	cactactagt	aaactgcagt	cctttgagga	tttagccagt	300
	atttctacct	atggctttcg	aggtgaggct	ttggccagca	taagccatgt	ggctcatgtt	360
	actattacaa	cgaaaacagc	tgatggaaaag	tgtgcataca	gagcaagtta	ctcagatgga	420
25	aaactgaaaag	cccctoctaa	accatgtgct	ggcaatcaag	ggacccagat	cacgggtggag	480
	gacctttttt	acaacatagc	cacgaggaga	aaagctttaa	aaaatccaag	tgaagaatat	540
	gggaaaattt	tggaaagttg	tggcaggtat	tcagtaacac	atgcaggcat	tagtttctca	600
	gttaaaaaaac	aaggagagac	agtagctgat	gttaggacac	tacccaatgc	ctcaaccgtg	660
	gacaatatct	gctccatctt	tggaaatgct	gttagtcgag	aactgataga	aattggatgt	720
30	gaggataaaa	ccctagcctt	caaaatgaat	ggttacatat	ccaatgcaaa	ctactcagt	780
	aagaagtgca	tcttcttact	cttcatcaac	catcgctctg	tagaatcaac	ttccttgaga	840
	aaagccatag	aaacagtgta	tgcagcctat	ttgccccaaa	acacacaccc	attcctgtac	900
	ctcagtttag	aaatcagtc	ccagaatgtg	gatgttaatg	tgcacccac	aaagcatgaa	960
	gttcacttcc	tgcacgagga	gagcatcctg	gagcgggtgc	agcagcacat	cgagagcaag	1020
35	ctcctgggct	ccaattcctc	caggatgtac	ttcaccacga	ctttgtacc	aggacttgct	1080
	ggccctctctg	gggagatggg	taaaaccaca	acaagtctga	cctcgtcttc	tacttttgga	1140
	agtagtgata	aggtctatgc	ccaccagatg	gttcgtacag	attcccgga	acagaagctt	1200
	gatgcatttc	tgcagcctct	gagcaaaccc	ctgtccagtc	agccccaggc	cattgtcaca	1260
	gaggataaga	cagatatttc	tagtggcagg	gctaggcagc	aagatgagga	gatgcttgaa	1320
40	ctcccagccc	ctgctgaagt	ggctgcaaaa	aatcagagct	tggaggggga	tacaacaaag	1380
	gggaccttcag	aaatgtcaga	gaagagagga	cctacttcca	gcaaccccag	aaagagacat	1440
	cgggaagatt	ctgatgtgga	aatgggtgga	gatgattccc	gaaaggaaat	gactgcagct	1500
	tgtaccccc	ggagaaggat	cattaacctc	actagtgttt	tgaagtctca	ggaagaaatt	1560
	aatgagcagg	gacatgaggt	tctccgggag	atgttgcata	accactcctt	cgtgggctgt	1620
45	gtgaatcctc	agtgggcctt	ggcacagcat	caaaccaagt	tataccttct	caacaccacc	1680
	aagcttagtg	aagaactgtt	ctaccagata	ctcatttatg	attttgccaa	ttttgggtgtt	1740
	ctcaggttat	cggagccagc	accgctcttt	gaccttgcca	tgcttgcttt	agatagtcca	1800
	gagagtggct	ggacagagga	agatgggtccc	aaagaaggac	ttgctgaata	cattgttgag	1860
	tttctgaaga	agaaggctga	gatgcttgca	gactatttct	ctttggaaat	tgatgaggaa	1920
50	gggaacctga	ttggattacc	ccttctgatt	gacaactatg	tgcccccttt	ggagggactg	1980
	cctatcttca	ttcttcgact	agccaactgag	gtgaattggg	acgaagaaaa	ggaatgtttt	2040
	gaaagcctca	gtaaagaatg	cgctatgttc	tattccatcc	ggaagcagta	catatctgag	2100
	gagtcgaccc	tctcaggcca	gcagagtga	gtgcctggct	ccattccaaa	ctcctggaag	2160
	tggactgtgg	aacacattgt	ctataaagcc	ttgcgctcac	acattctgcc	tcctaaacat	2220
55	ttcacagaag	atggaaatat	cctgcagctt	gctaacctgc	ctgatctata	caaagtcttt	2280
	gagagggtgt	aaatatgtgt	atttatgcac	tgtgggatgt	gttcttcttt	ctctgtattc	2340
	cgatacaaag	tgttgtatca	aagtgtgata	tacaaagtgt	accaacataa	gtgttggtag	2400
	cacttaagac	ttatacttgc	cttctgatag	tattccttta	tacacagtgg	attgattata	2460
60	aataaaataga	tgtgtcttaa	cata				2484

hPMS2-134 (human) (SEQ ID NO:15)

MERAESSSTE PAKAIKPIDR KSVHQICSGQ VVLSLSTAVK ELVENSIDAG ATNIDLKLKD 60
 YGVDLIEVSD NGCGVEEENF EGLTLKHHTS KIQEFADLTQ VETFGFRGEA LSSLCALSDV 120
 TISTCHASAK VGT 133

5 hPMS2-134 (human cDNA) (SEQ ID NO:16)

cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtac agaacctgct 60
 aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcaggtggta 120
 ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tgggtccact 180
 aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtctc agacaatgga 240
 10 tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300
 caagagtttg ccgacctaac tcaggttgaa acttttggct ttcgggggga agctctgagc 360
 tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga 420
 acttga 426

15 For further information on the background of the invention the following
 references may be consulted, each of which is incorporated herein by reference in its
 entirety:

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The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLE 1: Stable expression of dominant negative MMR genes in hybridoma cells

It has been previously shown by Nicolaides *et al.* (Nicolaides *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype *Mol. Cell. Biol.* 18:1635-1641) that the expression of a dominant negative allele in an otherwise MMR proficient cell could render these host cells MMR deficient. The creation of MMR deficient cells can lead to the generation of genetic alterations

throughout the entire genome of a host organisms offspring, yielding a population of genetically altered offspring or siblings that may produce biochemicals with altered properties. This patent application teaches of the use of dominant negative MMR genes in antibody-producing cells, including but not limited to rodent hybridomas, human
5 hybridomas, chimeric rodent cells producing human immunoglobulin gene products, human cells expressing immunoglobulin genes, mammalian cells producing single chain antibodies, and prokaryotic cells producing mammalian immunoglobulin genes or chimeric immunoglobulin molecules such as those contained within single-chain antibodies. The cell expression systems described above that are used to produce
10 antibodies are well known by those skilled in the art of antibody therapeutics.

To demonstrate the ability to create MMR defective hybridomas using dominant negative alleles of MMR genes, we first transfected a mouse hybridoma cell line that is known to produce and antibody directed against the human IgE protein with an expression vector containing the human PMS2 (cell line referred to as HBPMS2), the previously
15 published dominant negative PMS2 mutant referred herein as PMS134 (cell line referred to as HB134), or with no insert (cell line referred to as HBvec). The results showed that the PMS134 mutant could indeed exert a robust dominant negative effect, resulting in biochemical and genetic manifestations of MMR deficiency. Unexpectedly was the finding that the full length PMS2 also resulted in a lower MMR activity while no effect
20 was seen in cells containing the empty vector. A brief description of the methods is provided below.

The MMR proficient mouse H36 hybridoma cell line was transfected with various *hPMS2* expression plasmids plus reporter constructs for assessing MMR activity. The MMR genes were cloned into the pEF expression vector, which contains
25 the elongation factor promoter upstream of the cloning site followed by a mammalian polyadenylation signal. This vector also contains the NEO^r gene that allows for selection of cells retaining this plasmid. Briefly, cells were transfected with 1 µg of each vector using polyliposomes following the manufacturer's protocol (Life Technologies). Cells were then selected in 0.5 mg/ml of G418 for 10 days and G418
30 resistant cells were pooled together to analyze for gene expression. The pEF construct contains an intron that separates the exon 1 of the EF gene from exon 2, which is juxtaposed to the 5' end of the polylinker cloning site. This allows for a rapid reverse transcriptase polymerase chain reaction (RT-PCR) screen for cells expressing the spliced

products. At day 17, 100,000 cells were isolated and their RNA extracted using the trizol method as previously described (Nicolaidis N.C., Kinzler, K.W., and Vogelstein, B. (1995) Analysis of the 5' region of PMS2 reveals heterogeneous transcripts and a novel overlapping gene. *Genomics* 29:329-334). RNAs were reverse transcribed using
5 Superscript II (Life Technologies) and PCR amplified using a sense primer located in exon 1 of the EF gene (5'-ttt cgc aac ggg ttt gcc g-3') and an antisense primer (5'-gtt tca gag tta agc ctt cg-3') centered at nt 283 of the published human PMS2 cDNA, which will detect both the full length as well as the PMS134 gene expression. Reactions were carried out using buffers and conditions as previously described (Nicolaidis, N.C., *et al.* (1995) Genomic organization of the human PMS2 gene family. *Genomics* 30:195-206), using the following amplification parameters: 94°C for 30 sec, 52°C for 2 min, 72°C for 2 min, for 30 cycles. Reactions were analyzed on agarose gels. Figure 1 shows a representative example of PMS expression in stably transduced H36 cells.

Expression of the protein encoded by these genes were confirmed via western
15 blot using a polyclonal antibody directed to the first 20 amino acids located in the N-terminus of the protein following the procedures previously described (data not shown) (Nicolaidis *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype. *Mol. Cell. Biol.* 18:1635-1641.

20 **EXAMPLE 2: hPMS134 Causes a Defect in MMR Activity and hypermutability in hybridoma cells**

A hallmark of MMR deficiency is the generation of unstable microsatellite repeats in the genome of host cells. This phenotype is referred to as microsatellite instability (MI) (Modrich, P. (1994) Mismatch repair, genetic stability, and cancer *Science* 266:1959-1960; Palombo, F., *et al.* (1994) Mismatch repair and cancer *Nature* 36:417).
25 MI consists of deletions and/or insertions within repetitive mono-, di- and/or tri nucleotide repetitive sequences throughout the entire genome of a host cell. Extensive genetic analysis eukaryotic cells have found that the only biochemical defect that is capable of producing MI is defective MMR (Strand, M., *et al.* (1993) Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair *Nature* 365:274-276; Perucho, M. (1996) Cancer of the microsatellite mutator phenotype. *Biol Chem.* 377:675-684; Eshleman J.R., and Markowitz, S.D. (1996) Mismatch repair defects in human carcinogenesis. *Hum. Mol. Genet.* 5:1489-494). In light of this unique feature
30

that defective MMR has on promoting MI, it is now used as a biochemical marker to survey for lack of MMR activity within host cells (Perucho, M. (1996) Cancer of the microsatellite mutator phenotype. *Biol Chem.* 377:675-684; Eshleman J.R., and Markowitz, S.D. (1996) Mismatch repair defects in human carcinogenesis. *Hum. Mol. Genet.* 5:1489-494; Liu, T., *et al.* (2000) Microsatellite instability as a predictor of a mutation in a DNA mismatch repair gene in familial colorectal cancer *Genes Chromosomes Cancer* 27:17-25).

A method used to detect MMR deficiency in eukaryotic cells is to employ a reporter gene that has a polynucleotide repeat inserted within the coding region that disrupts its reading frame due to a frame shift. In the case where MMR is defective, the reporter gene will acquire random mutations (i.e. insertions and/or deletions) within the polynucleotide repeat yielding clones that contain a reporter with an open reading frame. We have employed the use of an MMR-sensitive reporter gene to measure for MMR activity in HBvec, HBPMS2, and HBPMS134 cells. The reporter construct used the pCAR-OF, which contains a hygromycin resistance (HYG) gene plus a β -galactosidase gene containing a 29 bp out-of-frame poly-CA tract at the 5' end of its coding region. The pCAR-OF reporter would not generate β -galactosidase activity unless a frame-restoring mutation (i.e., insertion or deletion) arose following transfection. HBvec, HBPMS2, and HB134 cells were each transfected with pCAR-OF vector in duplicate reactions following the protocol described in Example 1. Cells were selected in 0.5 mg/ml G418 and 0.5mg/ml HYG to select for cells retaining both the MMR effector and the pCAR-OF reporter plasmids. All cultures transfected with the pCAR vector resulted in a similar number of HYG/G418 resistant cells. Cultures were then expanded and tested for β -galactosidase activity *in situ* as well as by biochemical analysis of cell extracts. For *in situ* analysis, 100,000 cells were harvested and fixed in 1% gluteraldehyde, washed in phosphate buffered saline solution and incubated in 1 ml of X-gal substrate solution [0.15 M NaCl, 1 mM MgCl₂, 3.3 mM K₄Fe(CN)₆, 3.3 mM K₃Fe(CN)₆, 0.2% X-Gal] in 24 well plates for 2 hours at 37°C. Reactions were stopped in 500 mM sodium bicarbonate solution and transferred to microscope slides for analysis. Three fields of 200 cells each were counted for blue (β -galactosidase positive cells) or white (β -galactosidase negative cells) to assess for MMR inactivation. Table 1 shows the results from these studies. While no β -galactosidase positive cells were observed in HBvec cells, 10% of the cells per

field were β -galactosidase positive in HB134 cultures and 2% of the cells per field were β -galactosidase positive in HBPMS2 cultures.

Cell extracts were prepared from the above cultures to measure β -galactosidase using a quantitative biochemical assay as previously described (Nicolaidis *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype *Mol. Cell. Biol.* 18:1635-1641; Nicolaidis, N.C., *et al.* (1992) The Jun family members, c-JUN and JUND, transactivate the human *c-myc* promoter via an Ap1 like element. *J. Biol. Chem.* 267:19665-19672). Briefly, 100,000 cells were collected, centrifuged and resuspended in 200 μ l of 0.25M Tris, pH 8.0. Cells were lysed by freeze/thawing three times and supernatants collected after microfugation at 14,000 rpm to remove cell debris. Protein content was determined by spectrophotometric analysis at OD²⁸⁰. For biochemical assays, 20 μ g of protein was added to buffer containing 45 mM 2-mercaptoethanol, 1mM MgCl₂, 0.1 M NaPO₄ and 0.6 mg/ml Chlorophenol red- β -D-galactopyranoside (CPRG, Boehringer Mannheim). Reactions were incubated for 1 hour, terminated by the addition of 0.5 M Na₂CO₃, and analyzed by spectrophotometry at 576 nm. H36 cell lysates were used to subtract out background. Figure 2 shows the β -galactosidase activity in extracts from the various cell lines. As shown, the HB134 cells produced the highest amount of β -galactosidase, while no activity was found in the HBvec cells containing the pCAR-OF. These data demonstrate the ability to generate MMR defective hybridoma cells using dominant negative MMR gene alleles.

Table 1. β -galactosidase expression of HBvec, HBPMS2 and HB134 cells transfected with pCAR-OF reporter vectors. Cells were transfected with the pCAR-OF β -galactosidase reporter plasmid. Transfected cells were selected in hygromycin and G418, expanded and stained with X-gal solution to measure for β -galactosidase activity (blue colored cells). 3 fields of 200 cells each were analyzed by microscopy. The results below represent the mean +/- standard deviation of these experiments.

produced by each clone. Reactions are stopped by adding 50 μ ls of 500mM sodium bicarbonate and analyzed by OD at 415nm using a BioRad plate reader. Clones exhibiting an enhanced signal over background cells (H36 control cells) are then isolated and expanded into 10 ml cultures for additional characterization and confirmation of ELISA data in triplicate experiments. ELISAs are also performed on conditioned (CM) from the same clones to measure total Ig production within the conditioned medium of each well. Clones that produce an increased ELISA signal and have increased antibody levels are then further analyzed for variants that over-express and/or over-secrete antibodies as described in Example 4. Analysis of five 96-well plates each from HBvec or HB134 cells have found that a significant number of clones with a higher Optical Density (OD) value is observed in the MMR-defective HB134 cells as compared to the HBvec controls. Figure 4 shows a representative example of HB134 clones producing antibodies that bind to specific antigen (in this case IgE) with a higher affinity. Figure 4 provides raw data from the analysis of 96 wells of HBvec (left graph) or HB134 (right graph) which shows 2 clones from the HB134 plate to have a higher OD reading due to 1) genetic alteration of the antibody variable domain that leads to an increased binding to IgE antigen, or 2) genetic alteration of a cell host that leads to over-production/secretion of the antibody molecule. Anti-Ig ELISA found that the two clones, shown in figure 4 have Ig levels within their CM similar to the surrounding wells exhibiting lower OD values. These data suggest that a genetic alteration occurred within the antigen binding domain of the antibody which in turn allows for higher binding to antigen.

Clones that produced higher OD values as determined by ELISA were further analyzed at the genetic level to confirm that mutations within the light or heavy chain variable region have occurred that lead to a higher binding affinity hence yielding to a stronger ELISA signal. Briefly, 100,000 cells are harvested and extracted for RNA using the Triazol method as described above. RNAs are reverse transcribed using Superscript II as suggested by the manufacturer (Life Technology) and PCR amplified for the antigen binding sites contained within the variable light and heavy chains. Because of the heterogeneous nature of these genes, the following degenerate primers are used to amplify light and heavy chain alleles from the parent H36 strain.

Light chain sense: 5'-GGA TTT TCA GGT GCA GAT TTT CAG-3' (SEQ ID NO:1)

Light chain antisense: 5'-ACT GGA TGG TGG GAA GAT GGA-3' (SEQ ID NO:2)

Heavy chain sense: 5'-A(G/T) GTN (A/C)AG CTN CAG (C/G)AG TC-3' (SEQ ID NO:3)

- 5 Heavy chain antisense: 5'-TNC CTT G(A/G)C CCC AGT A(G/A)(A/T)C-3' (SEQ ID NO:4)

PCR reactions using degenerate oligonucleotides are carried out at 94°C for 30 sec, 52°C for 1 min, and 72°C for 1 min for 35 cycles. Products are analyzed on agarose gels.

10 Products of the expected molecular weights are purified from the gels by Gene Clean (Bio 101), cloned into T-tailed vectors, and sequenced to identify the wild type sequence of the variable light and heavy chains. Once the wild type sequence has been determined, non-degenerate primers were made for RT-PCR amplification of positive HB134 clones. Both the light and heavy chains were amplified, gel purified and sequenced using the

15 corresponding sense and antisense primers. The sequencing of RT-PCR products gives representative sequence data of the endogenous immunoglobulin gene and not due to PCR induced mutations. Sequences from clones were then compared to the wild type sequence for sequence comparison. An example of the ability to create *in vivo* mutations within an immunoglobulin light or heavy chain is shown in figure 5, where HB134 clone92 was

20 identified by ELISA to have an increased signal for hIgE. The light chain was amplified using specific sense and antisense primers. The light chain was RT-PCR amplified and the resulting product was purified and analyzed on an automated ABI377 sequencer. As shown in clone A, a residue -4 upstream of the CDR region 3 had a genetic change from ACT to TCT, which results in a Thr to Ser change within the framework region just

25 preceding the CDR#3. In clone B, a residue -6 upstream of the CDR region had a genetic change from CCC to CTC, which results in a Pro to His change within framework region preceding CDR#2.

The ability to generate random mutations in immunoglobulin genes or chimeric immunoglobulin genes is not limited to hybridomas. Nicolaides et al. (Nicolaides *et al.*

30 (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype *Mol. Cell. Biol.* 18:1635-1641) has previously shown the ability to generate hypermutable hamster cells and produce mutations within an endogenous gene. A common method for producing humanized antibodies is to graft CDR sequences from a MAAb

(produced by immunizing a rodent host) onto a human Ig backbone, and transfection of the chimeric genes into Chinese Hamster Ovary (CHO) cells which in turn produce a functional Ab that is secreted by the CHO cells (Shields, R.L., *et al.* (1995) Anti-IgE monoclonal antibodies that inhibit allergen-specific histamine release. *Int. Arch. Allergy Immunol.* 107:412-413). The methods described within this application are also useful for generating genetic alterations within Ig genes or chimeric Igs transfected within host cells such as rodent cell lines, plants, yeast and prokaryotes (Frigerio L, *et al.* (2000) Assembly, secretion, and vacuolar delivery of a hybrid immunoglobulin in plants. *Plant Physiol.* 123:1483-1494).

These data demonstrate the ability to generate hypermutable hybridomas, or other Ig producing host cells that can be grown and selected, to identify structurally altered immunoglobulins yielding antibodies with enhanced biochemical properties, including but not limited to increased antigen binding affinity. Moreover, hypermutable clones that contain missense mutations within the immunoglobulin gene that result in an amino acid change or changes can be then further characterized for *in vivo* stability, antigen clearance, on-off binding to antigens, etc. Clones can also be further expanded for subsequent rounds of *in vivo* mutations and can be screened using the strategy listed above.

The use of chemical mutagens to produce genetic mutations in cells or whole organisms are limited due to the toxic effects that these agents have on "normal" cells. The use of chemical mutagens such as MNU in MMR defective organisms is much more tolerable yielding to a 10 to 100 fold increase in genetic mutation over MMR deficiency alone (Bignami M, (2000) Unmasking a killer: DNA O(6)-methylguanine and the cytotoxicity of methylating agents. *Mutat. Res.* 462:71-82). This strategy allows for the use of chemical mutagens to be used in MMR-defective Ab producing cells as a method for increasing additional mutations within immunoglobulin genes or chimeras that may yield functional Abs with altered biochemical properties such as enhanced binding affinity to antigen, etc.

Example 4: Generation of antibody producing cells with enhanced antibody production

Analysis of clones from H36 and HB134 following the screening strategy listed above has identified a significant number of clones that produce enhanced amounts of antibody into the medium. While a subset of these clones gave higher Ig binding data as determined by ELISA as a consequence of mutations within the antigen binding domains contained in the variable regions, others were found to contain "enhanced" antibody production. A summary of the clones producing enhanced amounts of secreted MAb is shown in TABLE 2, where a significant number of clones from HB134 cells were found to produce enhanced Ab production within the conditioned medium as compared to H36 control cells.

TABLE 2. Generation of hybridoma cells producing high levels of antibody. HB134 clones were assayed by ELISA for elevated Ig levels. Analysis of 480 clones showed that a significant number of clones had elevated MAb product levels in their CM. Quantification showed that several of these clones produced greater than 500ngs/ml of MAb due to either enhanced expression and/or secretion as compared to clones from the H36 cell line.

Table 2. Production of MAb in CM from H36 and HB134 clones.

Cell Line	% clones > 400 ng/ml	% clones >500 ng/ml
H36	1/480 = 0.2%	0/480 = 0%
HB134	50/480 = 10%	8/480 = 1.7%

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Cellular analysis of HB134 clones with higher MAb levels within the conditioned medium (CM) were analyzed to determine if the increased production was simply due to genetic alterations at the Ig locus that may lead to over-expression of the polypeptides forming the antibody, or due to enhanced secretion due to a genetic alteration affecting secretory pathway mechanisms. To address this issue, we expanded three HB134 clones that had increased levels of antibody within their CM. 10,000 cells were prepared for western blot analysis to assay for intracellular steady state Ig protein levels (Figure 6). In addition, H36 cells were used as a standard reference (Lane 2) and a rodent fibroblast (Lane 1) was used as an Ig negative control. Briefly, cells were pelleted by centrifugation and lysed directly in 300 μ l of SDS lysis buffer (60 mM Tris, pH 6.8, 2% SDS, 10% glycerol,

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0.1 M 2-mercaptoethanol, 0.001% bromophenol blue) and boiled for 5 minutes. Lysate proteins were separated by electrophoresis on 4-12% NuPAGE gels (for analysis of Ig heavy chain. Gels were electroblotted onto Immobilon-P (Millipore) in 48 mM Tris base, 40 mM glycine, 0.0375% SDS, 20% methanol and blocked at room temperature for 1 hour
5 in Tris-buffered saline (TBS) plus 0.05% Tween-20 and 5% condensed milk. Filters were probed with a 1:10,000 dilution of sheep anti-mouse horseradish peroxidase conjugated monoclonal antibody in TBS buffer and detected by chemiluminescence using Supersignal substrate (Pierce). Experiments were repeated in duplicates to ensure reproducibility. Figure 6 shows a representative analysis where a subset of clones had enhanced Ig
10 production which accounted for increased Ab production (Lane 5) while others had a similar steady state level as the control sample, yet had higher levels of Ab within the CM. These data suggest a mechanism whereby a subset of HB134 clones contained a genetic alteration that in turn produces elevated secretion of antibody.

The use of chemical mutagens to produce genetic mutations in cells or whole
15 organisms are limited due to the toxic effects that these agents have on "normal" cells. The use of chemical mutagens such as MNU in MMR defective organisms is much more tolerable yielding to a 10 to 100 fold increase in genetic mutation over MMR deficiency alone (Bignami M, (2000) Unmasking a killer: DNA O(6)-methylguanine and the cytotoxicity of methylating agents. *Mutat. Res.* 462:71-82). This strategy allows for the
20 use of chemical mutagens to be used in MMR-defective Ab producing cells as a method for increasing additional mutations within immunoglobulin genes or chimeras that may yield functional Abs with altered biochemical properties such as enhanced binding affinity to antigen, etc.

25 **Example 5: establishment of genetic stability in hybridoma cells with new output trait.**

The initial steps of MMR are dependent on two protein complexes, called MutS α and MutL α (Nicolaidis *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype. *Mol. Cell. Biol.* 18:1635-1641). Dominant
30 negative MMR alleles are able to perturb the formation of these complexes with downstream biochemicals involved in the excision and polymerization of nucleotides comprising the "corrected" nucleotides. Examples from this application show the ability of a truncated MMR allele (PMS134) as well as a full length human PMS2 when expressed in

a hybridoma cell line is capable of blocking MMR resulting in a hypermutable cell line that gains genetic alterations throughout its entire genome per cell division. Once a cell line is produced that contains genetic alterations within genes encoding for an antibody, a single chain antibody, over expression of immunoglobulin genes and/or enhanced secretion of antibody, it is desirable to restore the genomic integrity of the cell host. This can be achieved by the use of inducible vectors whereby dominant negative MMR genes are cloned into such vectors, introduced into Ab producing cells and the cells are cultured in the presence of inducer molecules and/or conditions. Inducible vectors include but are not limited to chemical regulated promoters such as the steroid inducible MMTV, tetracycline regulated promoters, temperature sensitive MMR gene alleles, and temperature sensitive promoters.

The results described above lead to several conclusions. First, expression of hPMS2 and PMS134 results in an increase in microsatellite instability in hybridoma cells. That this elevated microsatellite instability is due to MMR deficiency was proven by evaluation of extracts from stably transduced cells. The expression of PMS134 results in a polar defect in MMR, which was only observed using heteroduplexes designed to test repair from the 5' direction (no significant defect in repair from the 3' direction was observed in the same extracts) (Nicolaidis *et al.* (1998) A Naturally Occurring hPMS2 Mutation Can Confer a Dominant Negative Mutator Phenotype. *Mol. Cell. Biol.* 18:1635-1641). Interestingly, cells deficient in hMLH1 also have a polar defect in MMR, but in this case preferentially affecting repair from the 3' direction (Drummond, J.T, *et al.* (1996) Cisplatin and adriamycin resistance are associated with MutLa and mismatch repair deficiency in an ovarian tumor cell line. *J. Biol. Chem.* 271:9645-19648). It is known from previous studies in both prokaryotes and eukaryotes that the separate enzymatic components mediate repair from the two different directions. Our results, in combination with those of Drummond *et al.* (Shields, R.L., *et al.* (1995) Anti-IgE monoclonal antibodies that inhibit allergen-specific histamine release. *Int. Arch Allergy Immunol.* 107:412-413), strongly suggest a model in which 5' repair is primarily dependent on hPMS2 while 3' repair is primarily dependent on hMLH1. It is easy to envision how the dimeric complex between PMS2 and MLH1 might set up this directionality. The combined results also demonstrate that a defect in directional MMR is sufficient to produce a MMR defective phenotype and suggests that any MMR gene allele is useful to produce genetically altered hybridoma cells, or a cell line that is producing Ig gene products. Moreover, the use of

such MMR alleles will be useful for generating genetically altered Ig polypeptides with altered biochemical properties as well as cell hosts that produce enhanced amounts of antibody molecules.

Another method that is taught in this application is that ANY method used to block MMR can be performed to generate hypermutability in an antibody-producing cell that can lead to genetically altered antibodies with enhanced biochemical features such as but not limited to increased antigen binding, enhanced pharmacokinetic profiles, etc. These processes can also be used to generate antibody producer cells that have increased Ig expression as shown in Example 4, figure 6 and/or increased antibody secretion as shown in Table 2.

In addition, we demonstrate the utility of blocking MMR in antibody producing cells to increase genetic alterations within Ig genes that may lead to altered biochemical features such as, but not limited to, increased antigen binding affinities (Figure 5A and 5B).

The blockade of MMR in such cells can be through the use of dominant negative MMR gene alleles from any species including bacteria, yeast, protozoa, insects, rodents, primates, mammalian cells, and man. Blockade of MMR can also be generated through the use of antisense RNA or deoxynucleotides directed to any of the genes involved in the MMR biochemical pathway. Blockade of MMR can be through the use of polypeptides that interfere with subunits of the MMR complex including but not limited to antibodies.

Finally, the blockade of MMR may be through the use chemicals such as but not limited to nonhydrolyzable ATP analogs, which have been shown to block MMR (Galio, L, *et al.* (1999) ATP hydrolysis-dependent formation of a dynamic ternary nucleoprotein complex with MutS and MutL. *Nucl. Acids Res.* 27:2325-23231).

WE CLAIM:

1. A method for making a hypermutable, antibody producing cell, comprising introducing into a cell capable of producing antibodies a polynucleotide comprising a dominant negative allele of a mismatch repair gene.
2. The method of claim 1 wherein said polynucleotide is introduced by transfection of a suspension of cells *in vitro*.
3. The method of claim 1 wherein said mismatch repair gene is *PMS2*.
4. The method of claim 1 wherein said mismatch repair gene is human *PMS2*.
5. The method of claim 1 wherein said mismatch repair gene is *MLH1*.
6. The method of claim 1 wherein said mismatch repair gene is *PMS1*.
7. The method of claim 1 wherein said mismatch repair gene is *MSH2*.
8. The method of claim 1 wherein said mismatch repair gene is *MSH2*.
9. The method of claim 4 wherein said allele comprises a truncation mutation.
10. The method of claim 4 wherein said allele comprises a truncation mutation at codon 134.
11. The method of claim 10 wherein said truncation mutation is a thymidine at nucleotide 424 of wild-type *PMS2*.
12. The method of claim 1 wherein said polynucleotide is introduced into a fertilized egg of an animal.
13. The method of claim 12 wherein the fertilized egg is subsequently implanted into a pseudo-pregnant female whereby the fertilized egg develops into a mature transgenic animal.
14. The method of claim 12 wherein said mismatch repair gene is *PMS2*.
15. The method of claim 12 wherein said mismatch repair gene is human *PMS2*.
16. The method of claim 12 wherein said mismatch repair gene is human *MLH1*.
17. The method of claim 12 wherein said mismatch repair gene is human *PMS1*.
18. The method of claim 11 wherein said mismatch repair gene is a human *mutL* homolog.
19. The method of claim 15 wherein said allele comprises a truncation mutation.
20. The method of claim 15 wherein said allele comprises a truncation mutation at codon 134.
21. The method of claim 19 wherein said truncation mutation is a thymidine at nucleotide 424 of wild-type *PMS2*.

22. The method of claim 1 wherein said capability is due to the co-introduction of an immunoglobulin gene into said cell.
23. A homogeneous culture of hypermutable, mammalian cells wherein said cells comprise a dominant negative allele of a mismatch repair gene.
24. The culture of hypermutable, mammalian cells of claim 23 wherein the mismatch repair gene is *PMS2*.
25. The culture of hypermutable, mammalian cells of claim 24 wherein the mismatch repair gene is human *PMS2*.
26. The culture of hypermutable, mammalian cells of claim 23 wherein the mismatch repair gene is *MLH1*.
27. The culture of hypermutable, mammalian cells of claim 23 wherein the mismatch repair gene is *PMS1*.
28. The culture of hypermutable, mammalian cells of claim 23 wherein the mismatch repair gene is a human *mutL* homolog.
29. The culture of hypermutable, mammalian cells of claim 23 wherein the cells express a protein consisting of the first 133 amino acids of hPMS2.
30. A method for generating a mutation in a gene affecting antibody production in an antibody-producing cell comprising:
 - growing a said cell comprising said gene and a dominant negative allele of a mismatch repair gene; and
 - testing the cell to determine whether said gene of interest harbors a mutation.
31. The method of claim 30 wherein the step of testing comprises analyzing a nucleotide sequence of said gene.
32. The method of claim 30 wherein the step of testing comprises analyzing mRNA transcribed from said gene.
33. The method of claim 30 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.
34. The method of claim 30 wherein the step of testing comprises analyzing the phenotype of said gene.
35. The method of claim 30 wherein the step of testing comprises analyzing the binding activity of an antibody.

36. A method wherein a mammalian cell is made MMR defective by the process of introducing a polynucleotide comprising an antisense oligonucleotide targeted against an allele of a mismatch repair gene into a mammalian cell, whereby the cell becomes hypermutable.
37. The method of claim 36 wherein the step of testing comprises analyzing a nucleotide sequence of said gene.
38. The method of claim 36 wherein the step of testing comprises analyzing mRNA transcribed from said gene.
39. The method of claim 36 wherein the step of testing comprises analyzing a protein encoded by said gene.
40. The method of claim 36 wherein the step of testing comprises analyzing the phenotype of said gene.
41. The method of claim 36 wherein the step of testing comprises analyzing the binding activity of an antibody.
42. A method for generating a mutation in a gene affecting antibody production in an antibody-producing cell comprising:
 - growing said cell comprising said gene and a polynucleotide encoding a dominant negative allele of a mismatch repair gene; and
 - testing said cell to determine whether said cell harbors at least one mutation in said gene yielding to a new biochemical feature to the product of said gene, wherein said new biochemical feature is selected from the group consisting of over-expression of said product, enhanced secretion of said product, enhanced affinity of said product for antigen, and combinations thereof.
43. The method of claim 42 wherein the step of testing comprises analyzing the steady state expression of the immunoglobulin gene of said cell.
44. The method of claim 42 wherein the step of testing comprises analyzing steady state mRNA transcribed from the immunoglobulin gene of said cell.
45. The method of claim 42 wherein the step of testing comprises analyzing the amount of secreted protein encoded by the immunoglobulin gene of said cell.
46. The method of claim 36 wherein the cell is made by the process of introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into a cell in the presence of DNA mutagens.

47. The method of claim 46 wherein the step of testing comprises analyzing a nucleotide sequence of an immunoglobulin gene of said cell.
48. The method of claim 46 wherein the step of testing comprises analyzing mRNA transcribed from the immunoglobulin gene of said cell.
49. The method of claim 46 wherein the step of testing comprises analyzing the immunoglobulin protein encoded by said gene.
50. The method of claim 46 wherein the step of testing comprises analyzing the biochemical activity of the protein encoded by said gene.
51. A hypermutable transgenic mammalian cell made by the method of claim 42.
52. The transgenic mammalian cell of claim 51 wherein said cell is from primate.
53. The transgenic mammalian cell of claim 51 wherein said cell is from rodent.
54. The transgenic mammalian cell of claim 51 wherein said cell is from human.
55. The transgenic mammalian cell of claim 51 wherein said cell is eukaryotic.
56. The transgenic mammalian cell of claim 51 wherein said cell is prokaryotic
57. A method of reversibly altering the hypermutability of an antibody producing cell comprising introducing an inducible vector into a cell, wherein said inducible vector comprises a dominant negative allele of a mismatch repair gene operably linked to an inducible promoter, and inducing said cell to express said dominant negative mismatch repair gene.
58. The method of claim 57 wherein said mismatch repair gene is *PMS2*.
59. The method of claim 58 wherein said mismatch repair gene is human *PMS2*.
60. The method of claim 57 wherein said mismatch repair gene is *MLH1*.
61. The method of claim 57 wherein said mismatch repair gene is *PMS1*.
62. The method of claim 57 wherein said mismatch repair gene is a human *mutL* homolog.
63. The method of claim 57 wherein said cell expresses a protein consisting of the first 133 amino acids of hPMS2.
64. The method of claim 57 further comprising analyzing the immunoglobulin protein expressed by said antibody producing cell.
65. The method of claim 64 further comprising ceasing induction of said cell, thereby restoring genetic stability of said cell.
66. A method of producing genetically altered antibodies comprising

transfecting a polynucleotide encoding an immunoglobulin protein into a cell, wherein said cell comprises a dominant negative mismatch repair gene;
growing said cell, thereby producing a hypermutated polynucleotide encoding a hypermutated immunoglobulin protein;
screening for a desirable property of said hypermutated immunoglobulin protein;
isolating said hypermutated polynucleotide; and
transfecting said hypermutated polynucleotide into a genetically stable cell, thereby producing a hypermutated antibody-producing, genetically stable cell.

67. The method of claim 66 wherein said mismatch repair gene is *PMS2*.
68. The method of claim 66 wherein said mismatch repair gene is human *PMS2*.
69. The method of claim 66 wherein said mismatch repair gene is *MLH1*.
70. The method of claim 66 wherein said mismatch repair gene is *PMS1*.
71. The method of claim 66 wherein said mismatch repair gene is a human *mutL* homolog.
72. The method of claim 66 wherein said cell expresses a protein consisting of the first 133 amino acids of hPMS2.

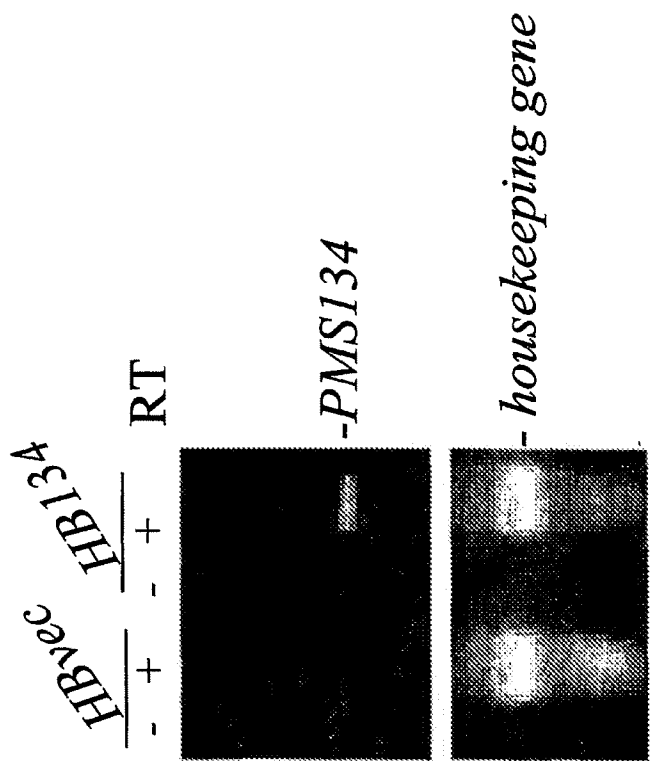


FIG. 1

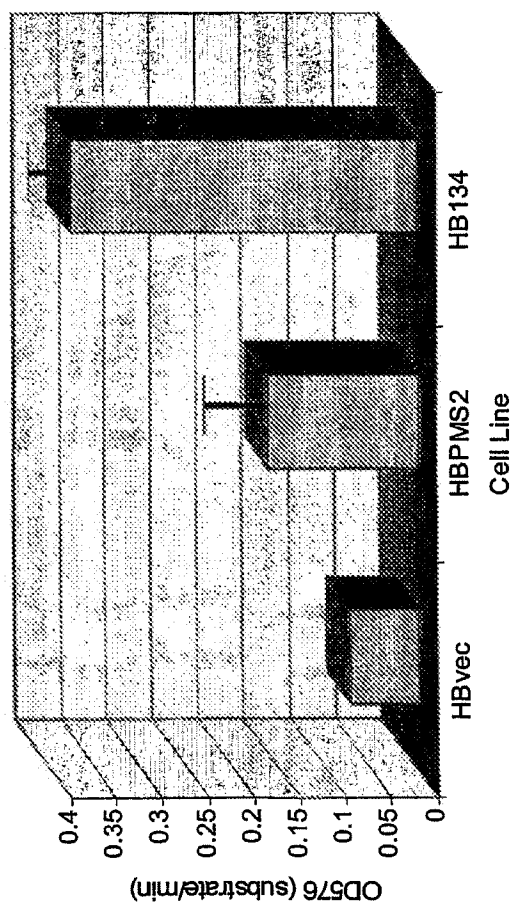
**Fig. 2**

Fig. 3

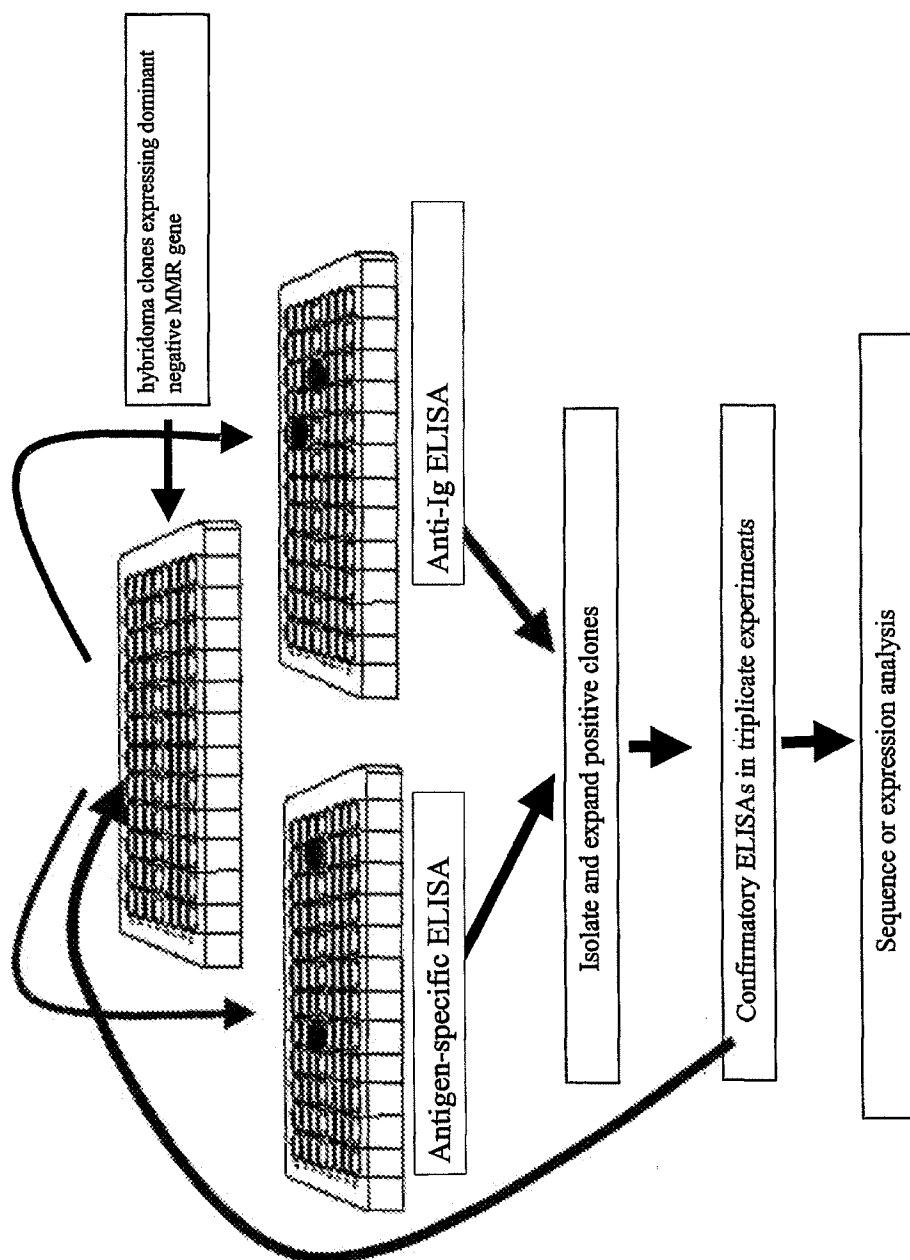
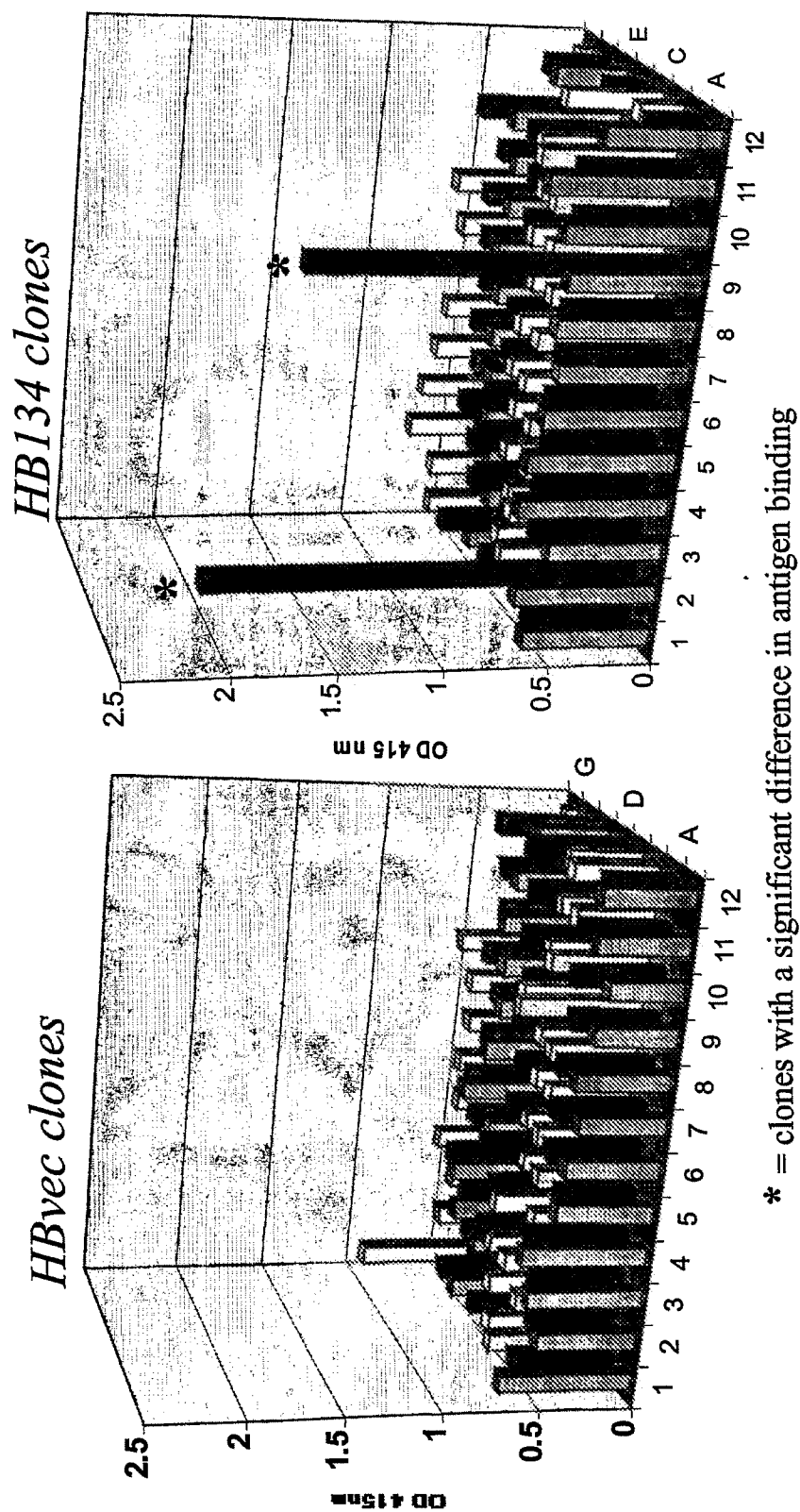


Fig.4



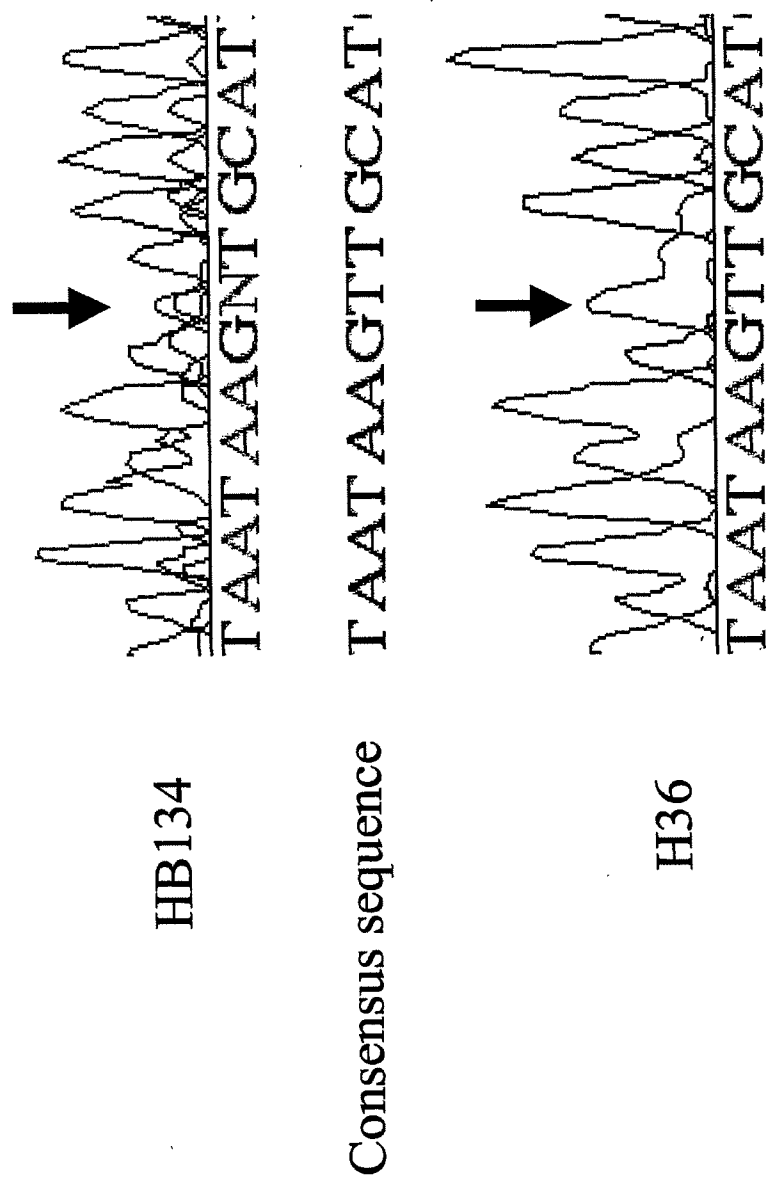


Fig. 5A

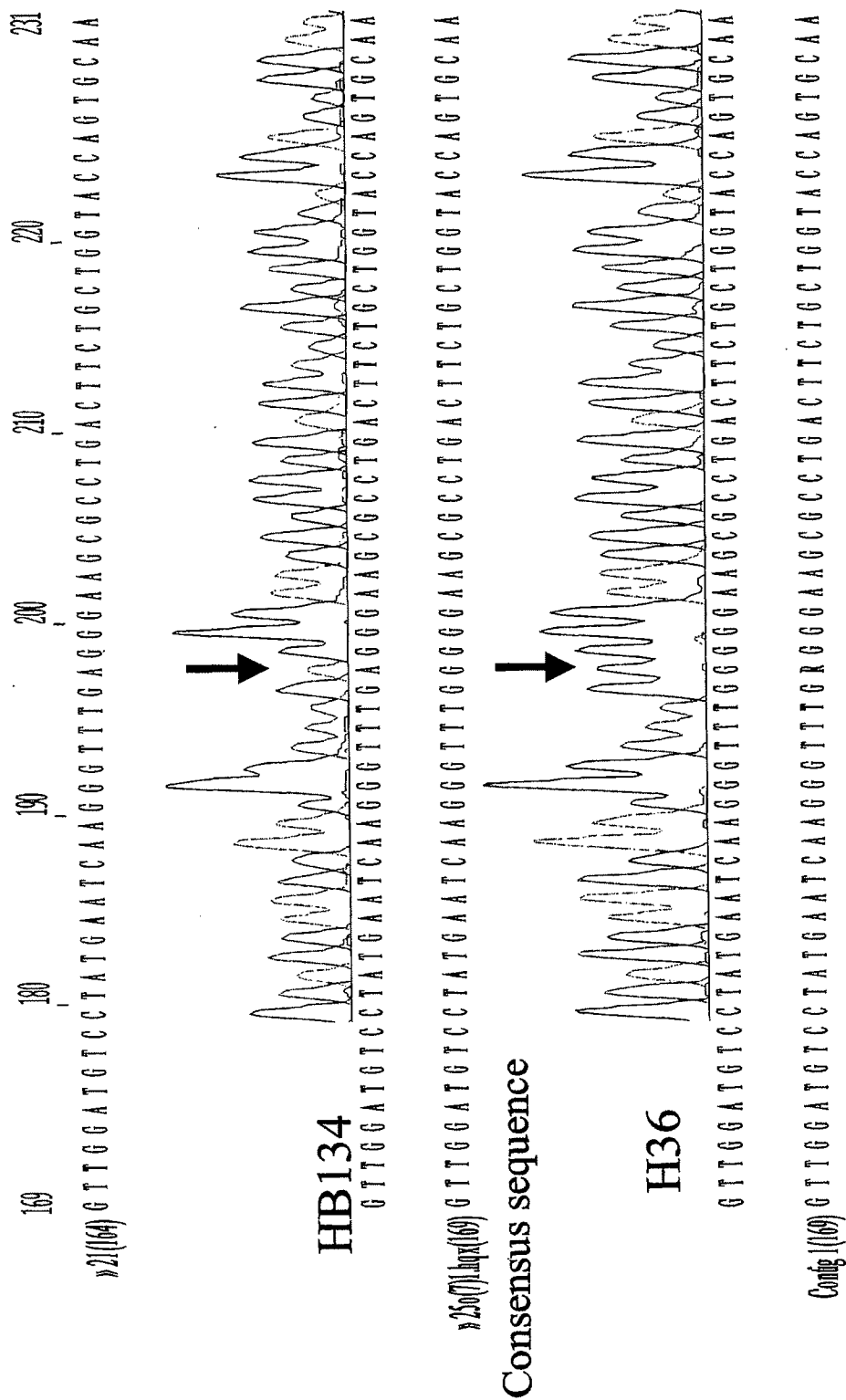


Fig. 5B

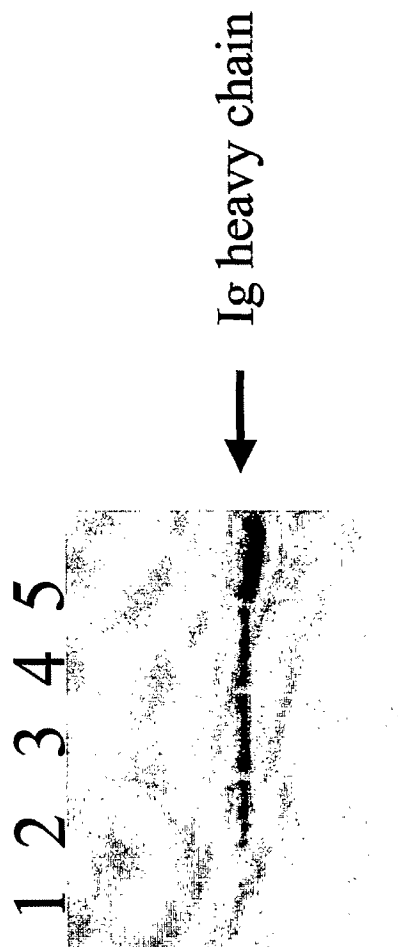


Fig. 6

SEQUENCE LISTING

<110> Nicolaides, Nicholas C
Grasso, Luigi
Sass, Philip M

<120> METHODS FOR GENERATING GENETICALLY ALTERED
ANTIBODY-PRODUCING CELL LINES WITH IMPROVED ANTIBODY
CHARACTERISTICS

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<141> 2000-11-07

<160> 16

<170> PatentIn Ver. 2.1

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Ala Gly Ala Thr Thr Ile Asp Leu Arg Leu Lys Asp Tyr Gly Val Asp
50 55 60

Leu Ile Glu Val Ser Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe
65 70 75 80

Glu Gly Leu Ala Leu Lys His His Thr Ser Lys Ile Gln Glu Phe Ala
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Asp Leu Thr Gln Val Glu Thr Phe Gly Phe Arg Gly Glu Ala Leu Ser

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Ala Ser Val Gly Thr Arg Leu Val Phe Asp His Asn Gly Lys Ile Thr		
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Gln Lys Thr Pro Tyr Pro Arg Pro Lys Gly Thr Thr Val Ser Val Gln		
145	150	155
His Leu Phe Tyr Thr Leu Pro Val Arg Tyr Lys Glu Phe Gln Arg Asn		
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Ile Lys Lys Glu Tyr Ser Lys Met Val Gln Val Leu Gln Ala Tyr Cys		
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Ile Ile Ser Ala Gly Val Arg Val Ser Cys Thr Asn Gln Leu Gly Gln		
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Gly Lys Arg His Ala Val Val Cys Thr Ser Gly Thr Ser Gly Met Lys		
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Asn Phe Glu Met Leu Gln Gln His Thr Val Leu Gln Ala Gln Arg Leu		
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Ile Thr Pro Gln Thr Leu Asn Leu Thr Ala Val Asn Glu Ala Val Leu		
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Asp Glu Asp Ala Pro Val Thr Glu Arg Ala Lys Leu Ile Ser Leu Pro		
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Thr Ser Lys Asn Trp Thr Phe Gly Pro Gln Asp Ile Asp Glu Leu Ile		
	770	775 780
Phe Met Leu Ser Asp Ser Pro Gly Val Met Cys Arg Pro Ser Arg Val		
785	790	795 800
Arg Gln Met Phe Ala Ser Arg Ala Cys Arg Lys Ser Val Met Ile Gly		
	805	810 815
Thr Ala Leu Asn Ala Ser Glu Met Lys Lys Leu Ile Thr His Met Gly		
	820	825 830
Glu Met Asp His Pro Trp Asn Cys Pro His Gly Arg Pro Thr Met Arg		
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His Val Ala Asn Leu Asp Val Ile Ser Gln Asn		
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Leu Ile Glu Val Ser Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe
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Glu Gly Leu Thr Leu Lys His His Thr Ser Lys Ile Gln Glu Phe Ala
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Ser Leu Cys Ala Leu Ser Asp Val Thr Ile Ser Thr Cys His Ala Ser
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Ala Lys Val Gly Thr Arg Leu Met Phe Asp His Asn Gly Lys Ile Ile
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Gln Leu Phe Ser Thr Leu Pro Val Arg His Lys Glu Phe Gln Arg Asn

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Gly Lys Arg Gln Pro Val Val Cys Thr Gly Gly Ser Pro Ser Ile Lys					
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Glu Asn Ile Gly Ser Val Phe Gly Gln Lys Gln Leu Gln Ser Leu Ile					
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	245		250		255
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Thr Pro Asp Lys Arg Gln Ile Leu Leu Gln Glu Glu Lys Leu Leu Leu					
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Ala Val Leu Lys Thr Ser Leu Ile Gly Met Phe Asp Ser Asp Val Asn					
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Lys Leu Asn Val Ser Gln Gln Pro Leu Leu Asp Val Glu Gly Asn Leu					
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Lys Arg Phe Lys Lys Glu Glu Ile Leu Ser Ser Ser Asp Ile Cys Gln		
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Lys Leu Val Asn Thr Gln Asp Met Ser Ala Ser Gln Val Asp Val Ala		
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Val Lys Ile Asn Lys Lys Val Val Pro Leu Asp Phe Ser Met Ser Ser		
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Leu Ala Lys Arg Ile Lys Gln Leu His His Glu Ala Gln Gln Ser Glu		
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Gly Glu Gln Asn Tyr Arg Lys Phe Arg Ala Lys Ile Cys Pro Gly Glu		
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Asn Gln Ala Ala Glu Asp Glu Leu Arg Lys Glu Ile Ser Lys Thr Met		
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Phe Ala Glu Met Glu Ile Ile Gly Gln Phe Asn Leu Gly Phe Ile Ile		

675	680	685
Thr Lys Leu Asn Glu Asp Ile Phe Ile Val Asp Gln His Ala Thr Asp		
690	695	700
Glu Lys Tyr Asn Phe Glu Met Leu Gln Gln His Thr Val Leu Gln Gly		
705	710	715
Gln Arg Leu Ile Ala Pro Gln Thr Leu Asn Leu Thr Ala Val Asn Glu		
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Ala Val Leu Ile Glu Asn Leu Glu Ile Phe Arg Lys Asn Gly Phe Asp		
740	745	750
Phe Val Ile Asp Glu Asn Ala Pro Val Thr Glu Arg Ala Lys Leu Ile		
755	760	765
Ser Leu Pro Thr Ser Lys Asn Trp Thr Phe Gly Pro Gln Asp Val Asp		
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Glu Leu Ile Phe Met Leu Ser Asp Ser Pro Gly Val Met Cys Arg Pro		
785	790	795
Ser Arg Val Lys Gln Met Phe Ala Ser Arg Ala Cys Arg Lys Ser Val		
805	810	815
Met Ile Gly Thr Ala Leu Asn Thr Ser Glu Met Lys Lys Leu Ile Thr		
820	825	830
His Met Gly Glu Met Asp His Pro Trp Asn Cys Pro His Gly Arg Pro		
835	840	845
Thr Met Arg His Ile Ala Asn Leu Gly Val Ile Ser Gln Asn		
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Phe Asp Lys Ile Glu Val Arg Asp Asn Gly Glu Gly Ile Lys Ala Val
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Asp Ala Pro Val Met Ala Met Lys Tyr Tyr Thr Ser Lys Ile Asn Ser
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His Glu Asp Leu Glu Asn Leu Thr Thr Tyr Gly Phe Arg Gly Glu Ala
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Leu Gly Ser Ile Cys Cys Ile Ala Glu Val Leu Ile Thr Thr Arg Thr
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 Thr Lys Asp Leu Glu Arg Tyr Asn Ser Gln Met Lys Arg Ala Ile Glu
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 Lys Asp Glu Pro Cys Leu Ile His Asn Leu Arg Phe Pro Asp Ala Trp
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 Glu Glu Ala Leu Leu Phe Lys Arg Leu Leu Glu Asn His Lys Leu Pro
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Ala Glu Pro Leu Glu Lys Pro Ile Met Leu Thr Glu Ser Leu Phe Asn
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Gly Ser His Tyr Leu Asp Val Leu Tyr Lys Met Thr Ala Asp Asp Gln
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Arg Tyr Ser Gly Ser Thr Tyr Leu Ser Asp Pro Arg Leu Thr Ala Asn
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Gly Phe Lys Ile Lys Leu Ile Pro Gly Val Ser Ile Thr Glu Asn Tyr
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Leu Glu Ile Glu Gly Met Ala Asn Cys Leu Pro Phe Tyr Gly Val Ala
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Asp Leu Lys Glu Ile Leu Asn Ala Ile Leu Asn Arg Asn Ala Lys Glu
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Val Tyr Glu Cys Arg Pro Arg Lys Val Ile Ser Tyr Leu Glu Gly Glu
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Ile Gln Asp Ile Ile Tyr Arg Met Lys His Gln Phe Gly Asn Glu Ile
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<211> 3063

<212> DNA

<213> Homo sapiens

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<211> 934

<212> PRT

<213> Homo sapiens

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Asp Ala Leu Leu Ala Ala Arg Glu Val Phe Lys Thr Gln Gly Val Ile
 50 55 60

Lys Tyr Met Gly Pro Ala Gly Ala Lys Asn Leu Gln Ser Val Val Leu
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Ser Lys Met Asn Phe Glu Ser Phe Val Lys Asp Leu Leu Leu Val Arg
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Gln Tyr Arg Val Glu Val Tyr Lys Asn Arg Ala Gly Asn Lys Ala Ser
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Lys Glu Asn Asp Trp Tyr Leu Ala Tyr Lys Ala Ser Pro Gly Asn Leu
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Val Gly Val Gly Tyr Val Asp Ser Ile Gln Arg Lys Leu Gly Leu Cys
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Glu Phe Pro Asp Asn Asp Gln Phe Ser Asn Leu Glu Ala Leu Leu Ile
 180 185 190

Gln Ile Gly Pro Lys Glu Cys Val Leu Pro Gly Gly Glu Thr Ala Gly
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Asp Met Gly Lys Leu Arg Gln Ile Ile Gln Arg Gly Gly Ile Leu Ile
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Thr Glu Arg Lys Lys Ala Asp Phe Ser Thr Lys Asp Ile Tyr Gln Asp

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Thr Gly Ser Gln Ser Leu Ala Ala Leu Leu Asn Lys Cys Lys Thr Pro						
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Pro Asp Leu Asn Arg Leu Ala Lys Lys Phe Gln Arg Gln Ala Ala Asn						
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Val Ile Gln Ala Leu Glu Lys His Glu Gly Lys His Gln Lys Leu Leu						
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<211> 3145

<212> DNA

<213> Homo sapiens

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<211> 756

<212> PRT

<213> Homo sapiens

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Gly Thr Gly Ile Arg Lys Glu Asp Leu Asp Ile Val Cys Glu Arg Phe
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Tyr Gly Phe Arg Gly Glu Ala Leu Ala Ser Ile Ser His Val Ala His
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 115 120 125

Ser Tyr Ser Asp Gly Lys Leu Lys Ala Pro Pro Lys Pro Cys Ala Gly
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Thr Arg Arg Lys Ala Leu Lys Asn Pro Ser Glu Glu Tyr Gly Lys Ile
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Leu Glu Val Val Gly Arg Tyr Ser Val His Asn Ala Gly Ile Ser Phe
 180 185 190

Ser Val Lys Lys Gln Gly Glu Thr Val Ala Asp Val Arg Thr Leu Pro
 195 200 205

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 225 230 235 240
 Lys Met Asn Gly Tyr Ile Ser Asn Ala Asn Tyr Ser Val Lys Lys Cys
 245 250 255
 Ile Phe Leu Leu Phe Ile Asn His Arg Leu Val Glu Ser Thr Ser Leu
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 Arg Lys Ala Ile Glu Thr Val Tyr Ala Ala Tyr Leu Pro Lys Asn Thr
 275 280 285
 His Pro Phe Leu Tyr Leu Ser Leu Glu Ile Ser Pro Gln Asn Val Asp
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 325 330 335
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 580 585 590
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 595 600 605
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30588

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : Please See Extra Sheet. US CL : 424/93.2, 130.1; 435 / 69.1, 410, 440; 536/24.5; 800/25 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 424/93.2, 130.1; 435 / 69.1, 410, 440; 536/24.5; 800/25 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN CAS, MEDLINE, BIOSIS, EMBASE, CAPLUS, WEST		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KONG, Q. et al. PMS2-deficiency diminishes hypermutation of a lambda ¹ transgene in young but not older mice. Molecular Immunology. 1999, Vol. 36, pages 83-91, entire document.	1-4, 12-15, 22-25, 30-56
Y	VORA K.A. et al. Severe Attenuation of the B Cell Immune Response In Msh2-deficient Mice. Journal of Experimental Medicine. February 1999, Vol. 189, No. 3, pages 471-481, entire document.	1-2, 7-8, 12-13, 22-23, 30-56.
Y	WINTER, D.B. ET AL. Altered spectra of hypermutation in antibodies from mice deficient for the DNA mismatch repair protein PMS2. Proc. Natl. Acad. Sci., USA. June 1998, Vol. 95, pages 6953-6958, entire document.	1-4, 12-15, 22-25, 30-56
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 05 JANUARY 2001	Date of mailing of the international search report 12 MAR 2001	
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer David Saunders Telephone No. (703) 308-0196	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/30588

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHRADER, C.E. et al. Reduced Isotype Switching in Splenic B Cells From Mice Deficient in Mismatch Repair Enzymes. Journal of Experimental Medicine. 1999, Vol. 190, No. 3, pages 323-330, entire document.	1-4, 6, 12-13, 17 22-23, 27, 30-56

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/30588

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

A01N 63/00; A61K 39/395, 48/00; C07H 21/04; C12N 5/00, 15/00; C12P 21/06